correlation between CPZeq and the each task performance or each NIRS measurement was found in the second group. For anxiolytics, there was no significant difference in task performances and NIRS measurements between administered versus non-administered subjects in the first group, except for the VFT category task performance ($t=2.481$, $P=.019$) where the patients that received anxiolytics showed a significantly lower score than those who didn't.

3.2. Linear discriminant analysis, a.k.a. LDA

3.2.1. Analysis of the task performances

All five tasks performance of VFT letter, VFT category, TOH, SBT and SRT was analyzed using a stepwise discriminant analysis procedure, which included performance of TOH, VFT letter and SBT as independent variables successively. This order of inclusion meant a higher contribution to the discrimination analysis of the diagnostic groups. These three variables correctly classified 83.3% of the first group subjects with a sensitivity of 80% and a specificity of 86.7% (i.e., 24 of the 30 schizophrenia patients and 26 of the 30 control subjects, respectively) (Wilks' $\lambda=0.499$, $P<.001$). As a prospective validation, the linear discriminant function derived from the first group was then used to classify data from the second group of subjects. In these subjects, 63.3% (i.e., 14 of the 30 schizophrenia patients and 24 of the 30 control subjects, respectively) were correctly classified, with a sensitivity of 46.7% and a specificity of 80% (Fig. 2).

3.2.2. NIRS measurements

Five Δ[oxy-Hb] activation measurements during the VFT letter, VFT category, TOH, SBT and SRT tasks were analyzed using a stepwise discriminant analysis procedure, which included Δ[oxy-Hb] of TOH, VFT letter and VFT category as independent variables successively. This order of inclusion meant a higher contribution to the discrimination analysis of the diagnostic groups. These three variables correctly classified 78.3% in the first group subjects with a sensitivity of 80% and a specificity of 76.7% (i.e., 24 of the 30 schizophrenia patients and 23 of the 30 control subjects, respectively) (Wilks' $\lambda=0.711$, $P<.001$). As a prospective validation, the linear discriminant function derived from the first group was used to classify data from the second group of subjects. In these subjects, 65% (i.e., 29 of the 30 schizophrenia patients and 10 of the 30 control subjects, respectively) were correctly classified, with a sensitivity of 96.7% and a specificity of 33.3% (Fig. 2).

3.2.3. Task performances plus NIRS measurements

Performances on all five tasks and Δ[oxy-Hb] measurements during the VFT letter, VFT category, TOH, SBT and SRT tasks were analyzed using a stepwise discriminant analysis procedure, which successively included task performance of TOH and VFT letter, Δ[oxy-Hb] of VFT letter and task performance of VFT category. This order of inclusion meant a higher contribution to the discrimination analysis of the diagnostic groups. These four variables correctly classified 88.3% in the first group, with a sensitivity of 80% and a

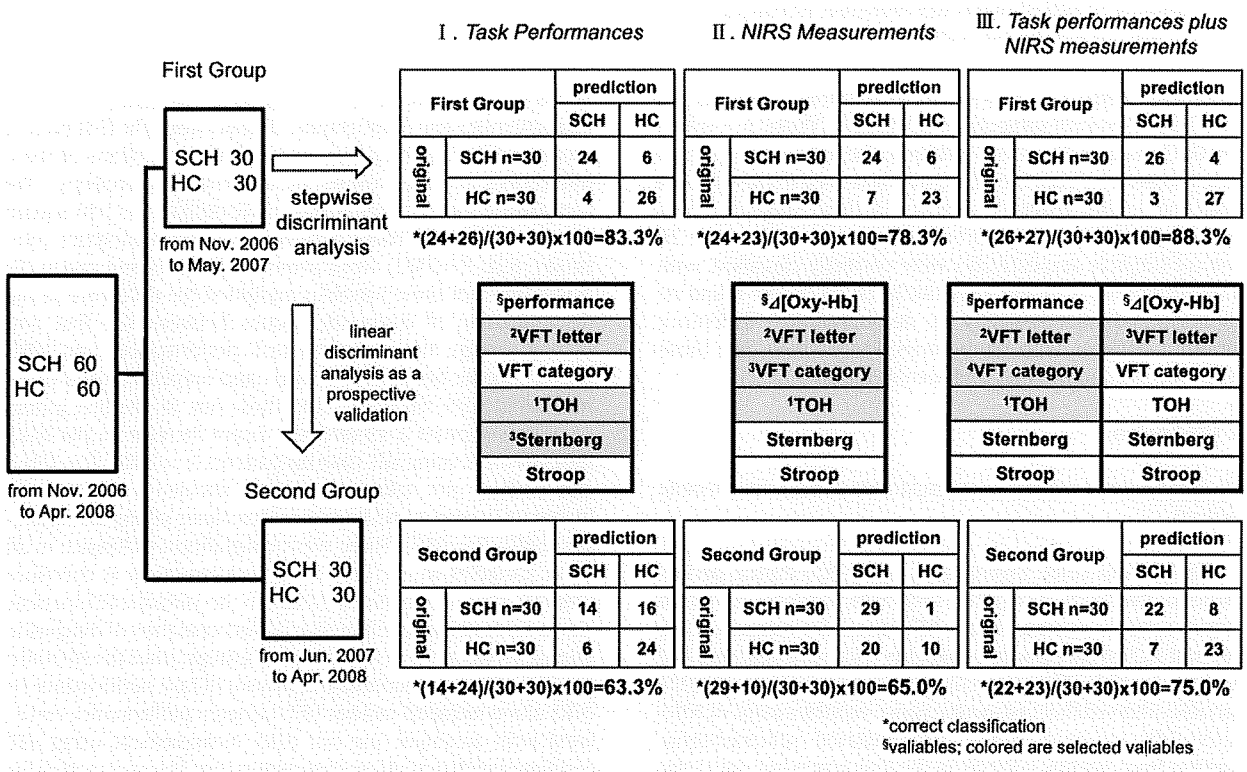

Fig. 2. Results.

Table 3
Recent studies for discriminant analysis in schizophrenia and non-patients with schizophrenia.

Article	Sample size	Variation study	Measure	Discrimination rate or sensitivity and specificity [a]
Physiological measures				
Kojima et al.	SCH = 145 HC = 124 Depression = 116	NA	Number of eye fixation Responsive search score	89.0% sensitivity 86.7% specificity
Matsushima et al.	SCH = 30 Non-SCH = 70 [b]	SCH = 30 Non-SCH[a] = 70	Exploratory eye movement	76.7% sensitivity 81.4% specificity
Morihisa et al.	SCH = 25 HC = 11	NA	Brain electrical activity mapping	95.0%
Neuropsychological batteries				
Arango et al.	SCH = 85 HC = 36	*NA*	Neuropsychological test battery composed of Category Fluency, Trail Making Test, Neurological Evaluation Scale	81.8%
Leontieva et al.	SCH = 62 HC = 80	NA	Pictogram test composed of Attribute Index, Geometric Index and Concrete Index.	91.0%
Midorikawa et al.	SCH = 27 HC = 49	NA	Finger movement test, pegboard, general memory, Attention and concentration, delayed recall, Full scale IQ, Wisconsin Card Sorting Test	96.1%
Functional brain imaging				
Levy et al.	SCH = 12 HC = 11	NA	Positron emission tomography, visual task	82–92%
Structural brain imaging				
Davatzikos et al.	SCH = 69 HC = 79	NA	Magnetic resonance imaging (MRI), Gray matter, white matter, and ventricular cerebrospinal fluid volumes	81.1%
Kawasaki et al.	SCH = 30 HC = 30	SCH = 16 HC = 16	MRI, gray matter	90%
Yoon et al.	SCH = 53 HC = 52	NA	MRI, cortical thickness of each lobe	88.8–93.6%

Abbreviations: SCH, schizophrenia; HC, healthy control; NA, not applicable.
[a] Data reflect discrimination rate unless otherwise stated.
[b] Consisted of 10 each of patients with depression, methamphetamine psychosis, alcohol psychosis, anxiety disorder, temporal lobe epilepsy, frontal lobe lesions as well as healthy normal controls.

specificity of 90% (i.e., 26 of the 30 schizophrenia patients and 27 of the 30 control subjects, respectively) (Wilks' $\lambda = 0.432$, $P < .001$). As a prospective validation, the linear discriminant function derived from the first group was used to classify data from the second group of subjects. In these subjects, 75.0% (i.e., 22 of the 30 schizophrenia patients and 23 of the 30 control subjects, respectively) were correctly classified, with a sensitivity of 73.3% and a specificity of 76.7%. We found no significant characteristically or demographical difference between patients who were correctly classified and those who were not, in the first and second group (Fig. 2).

4. Discussion

4.1. Discriminant analysis in schizophrenia patients and healthy subjects

The present study investigated Δ[oxy-Hb] during performance of five kinds of cognitive tasks which involve activation of the prefrontal cortex, namely VFT letter, VFT category, TOH, SBT and SRT, in two groups of patients with schizophrenia and healthy controls. The discriminant analysis with applicable variables showed that task performance variables alone or NIRS Δ[oxy-Hb] variables alone differentiated schizophrenia patients from healthy subjects in the first group but not in the second group. To elucidate such discrepancy in discrimination power of task performance or NIRS alone, we compared clinical characteristics and demographic data between the first patient group and the second group, and examine the effects of these differences on the results of discriminant analysis. The comparison showed significantly more outpatients (chi-square test, $P = .039$) and more patients receiving anxiolytics (chi-square test, $P = .002$) were present in the second group. To assess whether these variables explained the difference in the discrimination of diagnostic groups between the first and second groups, we compared task performances and NIRS measurements between inpatients and outpatients and those medicated with anxiolytics and those not. In the first group, inpatients showed a significantly larger increase in the NIRS measurement using SBT than outpatients ($t = 2.267$, $P = .031$), and patients not taking anxiolytics showed a significantly higher VFT category performance than those taking anxiolytics ($t = 2.481$, $P = .019$). There was no significant difference in all other variables in the first or second groups. It is therefore unlikely that anxiolytic medication or outpatient/inpatient status account for the difference in discrimination of diagnostic groups between the first and second groups, since the stepwise method of linear discriminant analyses of task performance or NIRS alone included neither VFT category performance correlated with anxiolytic use nor NIRS measurement using SBT correlated with outpatient/inpatient status. The explanation for this difference is therefore unknown.

Higher accuracy of discrimination was demonstrated in both first and second groups by the use of both task performance and NIRS variables. In this analysis, the significant independent variables were VFT letter (NIRS [oxy-Hb]), TOH, VFT letter and VFT category (performance). These results suggest that the combination of NIRS measurements and task performances as biological markers is more desirable for clinical application than NIRS or task performances alone.

The use of biological markers as potential diagnostic criteria depends on their ability to discriminate between patients with schizophrenia and non-patients with schizophrenia in a sensitive and specific way. Previous studies have investigated discrimination of patients with schizophrenia from non-patients with schizophrenia using methods such as physiological measures, cognitive batteries, functional and structural brain imaging (Table 3). Most of these studies have demonstrated higher than 80% sensitivity and specificity. However, only two studies, Kawasaki et al. (2007) and Matsushima et al. (1998), have investigated prospective validation. In the present study we have demonstrated prospective validation and have shown that using a combination of NIRS measurements and certain cognitive tasks provides the best classification rate. This suggests that combining tools from multiple fields will allow the development of better biological marker, possibly reflecting the pleiotropic or multifaceted aspects of schizophrenia. Indeed, while we have demonstrated that the combination of Δ[oxy-Hb] and task performance yielded acceptable results in the prospective validation, further studies could utilize simultaneous evaluation of additional disease characteristics including structural and functional brain imaging, physiological measurements, biochemical examination, neuropsychological batteries as well as psychopathology. This should provide even clearer diagnostic discrimination by taking into account the varied bio-psycho-social background of each schizophrenia case. NIRS represents an excellent physiological tool in this aim due to its advantages in efficiency, inspection time and limited invasiveness.

4.2. Limitations

A few limitations of the present study must be taken into account. Firstly, we could not thoroughly rule out the effect of antipsychotic medication taken by patients on prefrontal activation measures, performances of employed tasks and the results of linear discriminant analyses. However, evidence suggests that antipsychotics do not significantly affect prefrontal activation (Goldberg and Weinberger, 1996) or even show slight cognitive benefits from newer antipsychotic drugs (Bilder et al., 2002). In the present study, there was significant correlation between CPZeq and performance of SRT in the first group. No significant correlation was found between CPZeq and other task performances or NIRS measurements in either group. Therefore, we guessed that antipsychotic medications did not affect the results of linear discriminant analyses since the performance of SRT was excluded by stepwise method and no correlation was found between anitpsychotics and each NIRS measurement.

Secondly, since this study included relatively chronic patients with schizophrenia, it is difficult to address the specificity of the present study. Further studies that include different sub-groups, for example first-episode schizophrenia patients, relatives of patients, populations at high-risk of developing schizophrenia, as well as bipolar disorder patients should be conducted to assess this specificity.

Thirdly, the two-channel NIRS system used in this study could not cover the entire brain surface but only the frontal region; hence we could not assess the contribution of other brain areas to executing the tasks employed in this study. Further studies using a multi-channel NIRS system may help elucidate this issue.

4.3. Conclusion

In summary, the findings of this study indicate that the combination of NIRS measurements and cognitive task performances were more effective than NIRS or task performances alone for differentiating patients with schizophrenia from healthy subjects in prospective validation. The independent variables contributing to the differentiation were Δ[oxy-Hb] of VFT letter and task performances of TOH, VFT letter and VFT category. Our findings suggest that further NIRS studies for clinical application to schizophrenia are warranted.

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Contributors

M.A. and M.I. designed the study and wrote the protocol and undertook the statistical analysis. M.A., K.I., R.K., T.N., M.F., K.O., Y.Y. and H.T. conducted data acquisition. M.A. and M.I. analyzed data. M.A. wrote the first draft of the manuscript. L.C. contributed to the editing of the final manuscript. All authors revised it critically for important intellectual content and have approved the final manuscript. M.I., R.I., R.H., H.K., and M.T. supervised the entire project.

Conflict of interest

None.

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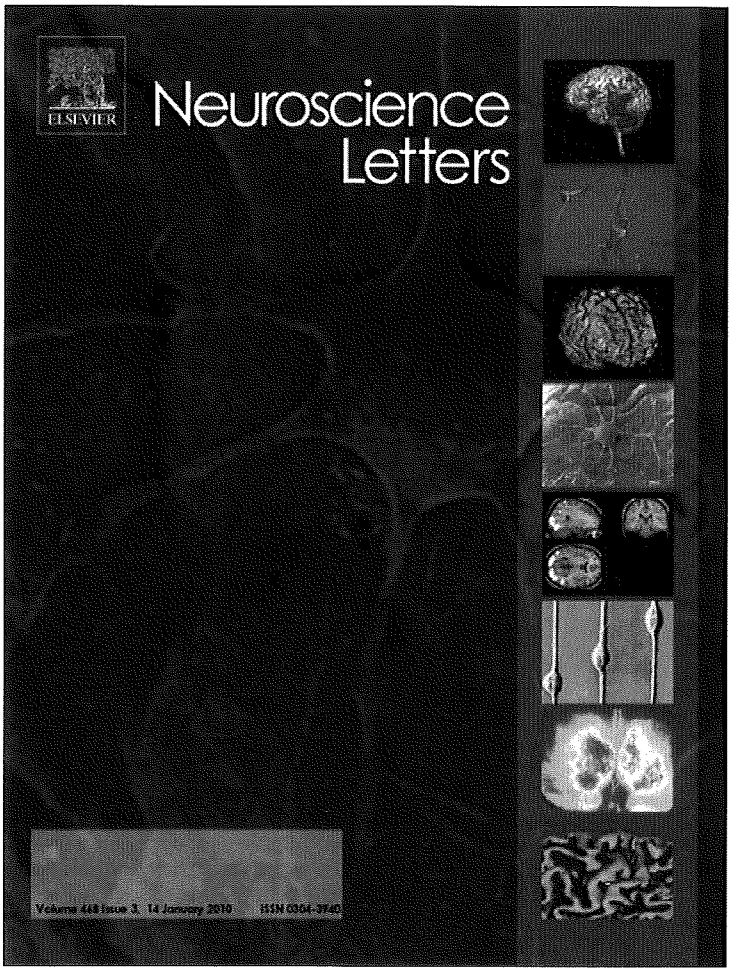

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Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder

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ABSTRACT

Pituitary adenylate cyclase-activating polypeptide (PACAP, ADCYAP1: adenylate cyclase-activating polypeptide 1) is a neuropeptide with neurotransmission modulating activity. The associations of the PACAP gene with schizophrenia and hippocampal volume have been reported. We recently reported depression-like behavior in the forced swimming test in PACAP deficient mice. Here we examined a possible association between the PACAP gene and major depressive disorder (MDD) in 637 patients and 967 controls and found that a genetic variant in the gene was associated with MDD. The present results suggest that PACAP signaling might contribute to the pathogenesis of MDD.

The adenylate cyclase-activating polypeptide 1 (ADCYAP1) gene encodes pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide originally isolated from ovine hypothalamus based on its ability to stimulate adenylate cyclase in rat anterior pituitary cell cultures. PACAP is a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon family. PACAP has been recognized as a pleiotropic neuropeptide that acts as a neurotransmitter, neuromodulator or neurotrophic factor through the activation of G-protein-linked receptors [5]. Mice lacking the PACAP gene exhibited marked behavioral abnormalities such as novelty-induced hyperactivity [6] and deficits in prepulse inhibition [16]. Further, we recently observed depression-like behavior in the forced swimming test in PACAP deficient mice [4], implying that PACAP is involved in fundamental mental processes.

Major depressive disorder (MDD) is a common and highly prevalent mental disorder with symptoms that include deficits in a range of cognitive, psychomotor and emotional processes. MDD is caused by a complex interaction of a large number of genetic (estimated heritability: 31–42%) and non-genetic factors, each with a relatively small contribution to the disorder [3]. The PACAP gene is located on 18p11, in which linkage studies have suggested as a locus for schizophrenia and bipolar disorder [13]. We recently reported the evidence for a possible association between PACAP signaling and schizophrenia [7]. In considering the emerging picture that major psychiatric disorders might share, at least in part, common genetic etiologies, it is plausible to assume that PACAP may be a risk factor for major mental illnesses, beyond schizophrenia. In the present study, we therefore pursued the possible association of the PACAP gene with MDD.

There were 637 patients with MDD [40.9% male, with a mean age of 51.3 years (SD 16.2) and mean age of onset of 46.2 years (SD 15.3)], and 967 healthy controls [47.7% male, with a mean age of 40.4 years (SD 16.1)]. All the subjects were biologically unrelated

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Table 1
Allele frequencies of seven SNPs in the PACAP gene (ADCYAP1) in patients with major depressive disorder and controls.

SNP-ID	dbSNP	Distance from SNP1	Major/minor polymorphism	Location	Number of subjects		Minor allele frequency		*P* value	Odds ratio (95% CI)
					Controls	Patients	Controls	Patients		
SNP1	rs2846584	–	C/T	5′-Region	967	637	0.362	0.362	0.972	
SNP2	rs2231181	712	G/C	5′-UTR	960	626	0.336	0.334	0.904	
SNP3	rs1893154	1071	G/A	Intron 1	951	633	*0.126*	*0.101*	*0.031*	*0.78(0.62–0.97)*
SNP4	rs1893153	1149	T/A	Intron 1	953	621	0.174	0.159	0.279	
SNP5	rs2856966	3656	A/G	Exon 3 (D54G)	953	630	0.047	0.063	0.058	1.35 (0.98–1.82)
SNP6	rs928978	4481	C/A	Intron 4	958	624	0.475	0.496	0.259	
SNP7	rs1610037	6581	A/G	3′-UTR	962	626	0.216	0.224	0.597	

Minor allele frequencies in controls are shown. CI: confidence interval. Significant results ($P < 0.05$) are italised.

Japanese. Patients were recruited at the National Center Hospital of Neurology and Psychiatry, Department of Psychiatry, Nagoya University Graduate School of Medicine, Department of Psychiatry, Fujita Health University School of Medicine, or Department of Psychiatry, Showa University School of Medicine. Healthy controls were recruited from local advertisements in Tokyo and Aichi, including hospital and institutional staffs. Consensus diagnosis was made for each patient by at least two trained psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV), based on unstructured clinical interviews and other available information including medical records and other research assessments. No patient was diagnosed by medical records alone. All patients were receiving treatment at the time that blood drawings were performed. Subjects with comorbid schizophrenia, bipolar disorder, or patients who had a history of substance abuse were excluded from the study. Controls were healthy volunteers who had no current or past contact with psychiatric services. After a description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethics committees and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. Seven single nucleotide polymorphisms (SNPs) in the PACAP gene were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, as described previously [7,9,10]. Primers and probes for detection of the SNPs are available upon request. Statistical analysis of genetic association studies was performed using SNPAlyse (DYNACOM, Yokohama, Japan). The presence of Hardy–Weinberg equilibrium was examined by using the χ^2-test for goodness of fit. Allele distributions between patients and controls were analyzed by the χ^2-test for independence. Statistical significance was defined as $P < 0.05$.

We have recently demonstrated an association between schizophrenia and the SNP3 (rs1893154) of the PACAP gene [7]. Therefore, in this study, we examined the possible association between this genetic variant and MDD. In total, 967 healthy controls and 637 patients with MDD were studied. The major allele of the SNP3 of the PACAP gene was in excess in patients with MDD ($\chi^2 = 4.7$, $P = 0.031$, odds ratio = 0.78, 95% confidence interval 0.62–0.97; Table 1). We also tested six other SNPs in the PACAP gene and found that SNP5 (rs2856966) tended to be associated with MDD, with a marginal *P* value of 0.058 (Table 1). The genotype distributions of all examined SNPs in the PACAP gene were in Hardy–Weinberg equilibrium for both the controls and patients with MDD ($P > 0.4$).

An association between MDD and the PACAP gene has been demonstrated for the first time in our sample of 637 patients and 967 healthy controls. We selected SNPs according to our previous association study between the PACAP gene and schizophrenia [7]. Our method of SNP selection was not a gene based method such as selection of Tagging SNPs. Five SNPs in the region of the PACAP gene (approximately 7.3 kb) were selected as Tag SNPs using SNPs consulted the HapMap database (www.hapmap.org, population: Japanese Tokyo) and Tagger program of the HAPLOVIEW software. Three out of five SNPs (SNP3, SNP5 and SNP7) was examined in our study. However, there is a possibility of genotyping more tagSNPs to fully understand the role of this gene in the etiology of MDD. The G/A SNP (SNP3) of the PACAP gene associated with the disorder is intronic, and so far, we could not detect whether this SNP influences the expression and/or function of the PACAP gene. However, the SNP3 has also been shown to have a possible impact on brain morphology. Homozygous carriers of the G allele showed smaller bilateral hippocampal volumes compared with A-carriers [7]. This is in line with a previous imaging study that has demonstrated that patients with a history of MDD have reduced hippocampal volumes [14]. The evidence for genetic associations with MDD (SNP3, odds ratio = 0.78; SNP5, odds ratio = 1.35) in the present study and with schizophrenia (SNP3, odds ratio = 0.74; SNP5, odds ratio = 1.38) in the previous study [7] suggests that the effect size of the contribution of the PACAP gene might be similar to both MDD and schizophrenia.

There are several limitations in our results. Lack of structured interview or standardized tests (e.g., Beck Depression Inventory) in patients could influence the results. As the huge heterogeneity was found in MDD, more detailed information for clinical variables in MDD was required such as severity of symptoms, with or without psychotic symptoms, pharmacoresistance, duration of illness, duration of treatment, family history of MDD and/or other psychiatric disorders, inpatient or outpatient, times of hospitalization, etc. However, we do not have the detail clinical information in our sample. These specific variables such as depressive symptom severity could be associated with the PACAP gene. As the PACAP SNP was also associated with schizophrenia, it may be more strongly associated with a subset of patients, for example, those with psychotic symptoms and/or reactivity to atypical antipsychotic medications [15]. Further studies differentiating MDD into various clinical subgroups are warranted in the future.

Altered HPA axis activity and cortisol secretion are commonly associated with MDD [17]. In addition, altered glucocorticoid receptor signaling has been implicated in this disorder [1]. Mice with forebrain-specific disruption of glucocorticoid receptors show increased depression-like behavior, such as increased immobility in the forced swim test. In mice lacking the PACAP gene, the circadian plasma corticosterone level was flattened, although overall corticosterone secretion was lower, instead of higher, than wild-type mice [4]. Glucocorticoid receptor mRNA levels were reduced in the hippocampi of PACAP deficient mice, however, the dexamethasone-induced suppression in corticosterone levels was normally seen [4]. The flattened circadian corticosterone level in these mutant mice might be consistent with studies demonstrating that depressed individuals exhibit a relatively flat and unresponsive pattern of cortisol secretion [2].

The finding that the PACAP gene might be associated with hippocampal volume [7] suggests that PACAP is involved in endophenotypes, such as impairments of neurophysiology of mental and cognitive processes, rather than being associated with specific psychiatric disorders. The pathophysiology of mental disorders can be a combination of subtle alterations of major signaling pathways, which are influenced by products of risk genes. A good example might be that the interaction between Disrupted-in-schizophrenia 1 (DISC1) and its binding protein, DISC1-Binding Zinc-finger protein, is involved in the PACAP signaling pathway [11]. DISC1 is a gene disrupted by a translocation that segregates with major psychiatric disorders including schizophrenia, bipolar disorder and MDD [12]. Subsequent study revealed that the DISC1 gene was associated with MDD [8]. Therefore, PACAP could be a part of a common genetic etiology shared by multiple mental disorders including MDD.

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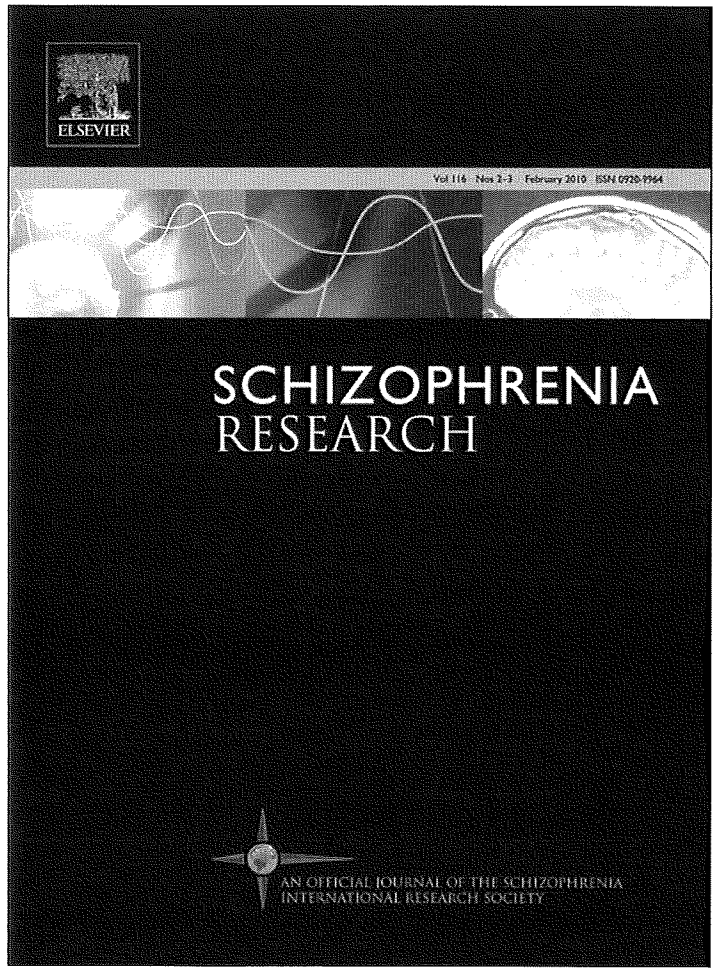

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The *chitinase 3-like 1* gene and schizophrenia: Evidence from a multi-center case–control study and meta-analysis

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ABSTRACT

The *chitinase 3-like 1* (*CHI3L1*) gene acts as a cellular survival factor in response to several environmental and psychosocial stresses. The expression level of *CHI3L1* was increased in the hippocampus and prefrontal cortex regions of patients with schizophrenia. Genetic variants of the *CHI3L1* gene have been significantly associated with schizophrenia in two distinct ethnic groups, the Chinese and Irish populations. The aims of this study are to confirm the association between the *CHI3L1* gene and schizophrenia in a Japanese population using the largest sample size to date (1463 cases and 1795 controls) and perform a meta-analysis of the combined samples (3005 cases, 3825 controls and 601 trios). We found significant associations between single nucleotide polymorphism (SNP) 4/rs4950928 ($p=0.009$), which is located in the promoter region of the *CHI3L1* gene, and haplotypes including this SNP and schizophrenia (the most significant global $p<0.001$). As the meta-analysis of the combined samples showed significant heterogeneity among studies of SNP3/rs10399805 ($p=0.026$) and SNP4 ($p<0.001$), we performed meta-analyses separately in the Japanese (2033 cases and 2365 controls) and Chinese populations (412 cases, 464 controls and 601 trios), the major groups analyzed in association studies of the *CHI3L1* gene. The meta-analysis in Japanese populations showed stronger evidence for the association of schizophrenia with SNP4 ($p=0.003$), while the meta-analysis in Chinese populations showed an association with a different variant (SNP3) ($p=0.003$). We conclude that the genetic variants in the *CHI3L1* gene have ethnic heterogeneity and confer a susceptibility to schizophrenia in Asian populations.

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1. Introduction

Schizophrenia (OMIM 181500) is a common and complex psychiatric disease. The lifetime morbidity rate is 0.5–1.0% across distinct populations. Family, twin and adoption studies of schizophrenia have indicated that there are strong genetic factors with an estimated heritability of approximately 80% (Cardno and Gottesman, 2000). Many genes have been implicated in the pathogenesis of schizophrenia (Sun et al., 2008).

The *chitinase 3-like 1* gene [*CHI3L1*, (OMIM 601525)] consists of 10 exons and spans approximately 8 kb of genomic DNA. The protein was named YKL-40 based on its three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L), and its molecular mass of 40 kDa (Johansen et al., 1992). The protein has several names, including chitinase 3-like 1, human cartilage glycoprotein-39 (HC gp39), breast regressing protein 39 (brp-39), 38-kDa heparin-binding glycoprotein (gp38k), chondrex and 40-kDa mammary gland protein (MGP-40). In this study, to avoid confounding these terms, the gene is referred to as *CHI3L1* and the protein is referred to as YKL-40.

This gene acts as a cellular survival factor in responses to a variety of adverse environments, including various types of physiologic stress, such as inflammation, hypoxia and nutrient deprivation. These stresses may induce high expression of *CHI3L1* (Junker et al., 2005; Recklies et al., 2005). YKL-40 is secreted by activated macrophages and neutrophils in different tissues during inflammation and during increased remodeling of the extracellular matrix (Kirkpatrick et al., 1995; Rehli et al., 1997; Volck et al., 1998). YKL-40 initiates mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI3K) signaling cascades in fibroblasts. Signaling leads to the phosphorylation of both the extracellular signal-regulated kinase (ERK)-1/2 MAP kinase- and the protein kinase B (AKT)-mediated signaling cascades, which are associated with the control of mitogenesis (Recklies et al., 2002). The PI3K pathway and the downstream phosphorylation of AKT in particular are strongly associated with cell survival (Bakkenist and Kastan, 2004), which suggests a role for YKL-40 as an anti-apoptotic protein.

The synthesis of YKL-40 is induced by the inflammatory cytokines IL-1, IL-6 and TNF-α (Ling and Recklies, 2004; Recklies et al., 2005; Johansen et al., 2006). The genetic variants of the *CHI3L1* gene and high serum levels of YKL-40 are associated with several inflammatory diseases, including sarcoidosis, asthma and inflammatory bowel diseases (Kruit et al., 2007; Kucur et al., 2007; Ober et al., 2008). The role of YKL-40 in the nervous system is unclear. YKL-40 is elevated in the cerebrospinal fluid (CSF) of patients with spinal diseases in which the neural tissue has been damaged or stressed, including cervical myelopathy, lumbar canal stenosis and lumbar disc herniation (Tsuji et al., 2002). High levels of YKL-40 in the CSF have also been reported in patients with purulent meningitis (Ostergaard et al., 2002). The *CHI3L1* gene expression analyses demonstrated higher postmortem mRNA levels in the hippocampus and prefrontal cortex of patients with schizophrenia than in the respective tissues of controls (Chung et al., 2003; Arion et al., 2007). It has been hypothesized that YKL-40 protects cells from undergoing apoptosis and plays a role in inflammatory processes in patients with schizophrenia.

The *CHI3L1* gene is located on chromosome 1q32.1 and shows evidence of modest linkage with schizophrenia (Shaw et al., 1998; Jang et al., 2007), although recent genome-wide association studies have not identified any variant of this gene that is associated with schizophrenia (O'Donovan et al., 2008). Zhao et al. (2007) have detected genetic associations between schizophrenia and three single nucleotide polymorphisms (SNPs; rs6691378, rs10399805 and rs4950928) within the promoter region of *CHI3L1* in two independent Chinese cohorts. They found that an allele at rs4950928 impaired MYC/MAX-regulated transcriptional activation of *CHI3L1* by altering the transcription factor consensus sequences. Yang et al. (2008) subsequently indicated significant associations between schizophrenia and two SNPs in an Irish cohort. One was the same SNP (rs10399805) in the promoter that was reported in the original study and the other SNP (rs2275351) was within the gene at intron 7. These findings suggest that the *CHI3L1* gene is likely involved in predisposition to schizophrenia. However, the two studies were not replicated in two more recent studies, one conducted with Chinese trio samples and Japanese case–control samples (Yamada et al., 2008) and the other studying a small Bulgarian population (Betcheva et al., 2009). To further investigate this controversial issue, we first investigated whether the *CHI3L1* gene is associated with schizophrenia in a large Japanese population. Second, we performed meta-analyses on the overall population and separately in Japanese and Chinese populations.

2. Methods

2.1. Subjects

The subjects in our genetic association study consisted of 1463 unrelated patients with schizophrenia [54.6% males (799/664), mean age ± SD; 47.3 ± 15.0 years] and 1795 unrelated healthy controls [51.3% males (920/875), mean age ± SD; 45.5 ± 20.1 years]. The sex ratio did not differ significantly between groups ($\chi^2 = 3.7$, $p = 0.06$), while the mean age differed significantly between groups ($z = -5.1$, $p < 0.001$). These subjects were independent of those used by Yamada et al. (2008). All subjects were biologically unrelated Japanese and were recruited at three geographic regions in Japan: Osaka, Aichi and Tokushima (Yamaguchi-Kabata et al., 2008; Ohi et al., 2009). Cases were recruited from both outpatients and inpatients at university hospitals and psychiatric hospitals. Each schizophrenic research subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria, based on an unstructured clinical interview. Controls, including the hospital and institutional staff, were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Fujita Health University, Nagoya University and Tokushima University.

2.2. SNP selection and SNP genotyping

We designed our replication study by selecting six SNPs in the *CHI3L1* gene and the flanking regions. Five of the six SNPs were identical to the SNPs used in the original study: rs2364574 (SNP1), rs6691378 (SNP2), rs10399805 (SNP3), rs4950928 (SNP4) and rs880633 (SNP5). The designations of these SNPs in parentheses are according to Zhao et al. (2007). The remaining SNP (rs2275351) was chosen from the following study as it showed evidence for association with schizophrenia (Yang et al., 2008). Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. These SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, California, USA), as described previously (Hashimoto et al., 2006, 2007; Ohi et al., 2009). Detailed information on the PCR conditions is available upon request. Genotyping call rates were 99.0% (SNP1), 95.0% (SNP2), 99.2% (SNP3), 99.6% (SNP4), 99.8% (SNP5) and 97.7% (rs2275351). SNP2 was excluded from the present study because this variant was not clearly discriminated as a result of a lower call rate. No deviation from Hardy–Weinberg equilibrium (HWE) in the examined SNPs was detected in the controls ($p>0.05$), while the genotypic frequencies of two SNPs deviated from HWE in the schizophrenia patients (SNP1; $p=0.016$, rs2275351; $p<0.001$). The positions of the five SNPs analyzed in the present study are indicated in Fig. 1.

2.3. Power analysis

We performed power calculations using the Power Calculator for Two Stage Association Studies [http://www.sph.umich.edu/csg/abecasis/CaTS/; (Skol et al., 2006)]. Power estimates were based on allele frequencies in patients ranging from 0.17 (SNP4) to 0.29 (SNP3), odds ratios ranging from 1.29 (SNP3) to 1.49 (SNP4) for each associated SNP, as indicated by Zhao et al. (2007), and an alpha level of 0.05. Power was calculated under a prevalence of 0.01 using a multiplicative model, assuming varying degrees of the marker allele frequency and the odds ratio.

2.4. Meta-analysis of the CHI3L1 association studies

The studies included in the meta-analysis were selected using the Schizophrenia Research Forum (http://www.schizophreniaforum.org) and PubMed with the search terms "CHI3L1" and "Schizophrenia." The analyzed data encompass all publications up to May 2009.

2.5. Statistical analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and SPSS 16.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were

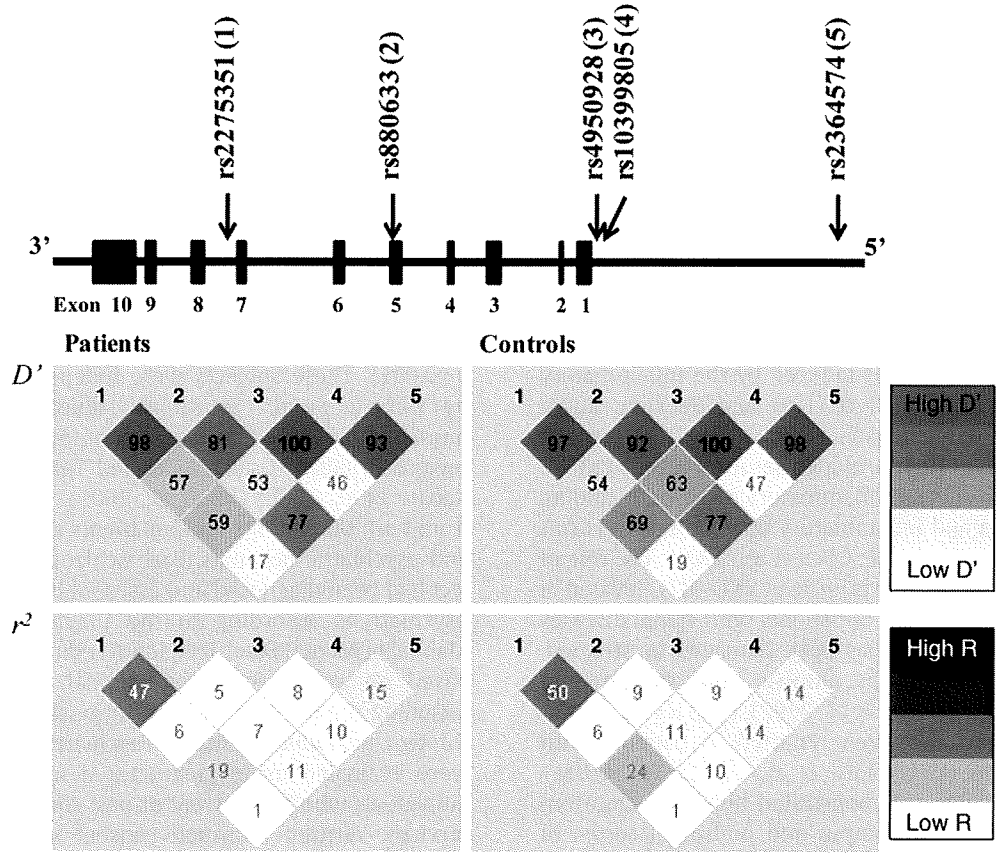

Fig. 1. Genomic structure of *CHI3L1*, including locations of the five SNPs studied, and linkage disequilibrium of these five SNPs in the patient and control groups. Based on an entry in the Entrez Gene database (National Center for Biotechnology Information), the genomic structure of *CHI3L1* is shown above. The locations of SNPs analyzed in this study are indicated by arrows. Numbers indicated in parentheses refer to numbering of the SNPs in the linkage disequilibrium (LD) diagram. The distances of exons–introns and intermarkers are drawn to scale. The LD between pairwise SNPs, using D' and r^2 values, are shown at the bottom of the map of gene structure separately for cases and controls. High levels of LD are represented by red (D') and black (r^2) coloring with increasing color intensity from 0 to 100, as shown by color bars.

analyzed using χ^2 tests for sex and the Mann–Whitney *U*-test for age. Deviation from HWE was tested separately in cases and controls using χ^2 tests for goodness of fit. The allelic and genotypic distributions of *CHI3L1* polymorphisms between patients and controls were analyzed using χ^2 tests. The number of effective independent SNPs assayed was estimated by the spectral decomposition method of Nyholt using SNPSpD software (Nyholt, 2004). Pairwise linkage disequilibrium (LD) analyses, expressed by D' and r^2, were applied to detect the intermarker relationship in each group using Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype frequencies were estimated by the method of maximum likelihood using genotyping data through the use of the expectation–maximization algorithm. Rare haplotypes found in less than 3% of both patients and controls were excluded from the haplotypic association analysis. We performed 10,000 permutations for most significant tests to determine empirical significance. We used a 2- to 5-window fashion analysis.

The meta-analyses were performed using the case–control and TDT meta-analysis package (catmap) for the R-project program (Version 2.8.0, http://www.r-project.org.), which implements fixed- and random-effect pooled estimates for case–control and the transmission disequilibrium method, allowing for the use of genetic association data across study types (Nicodemus, 2008). Cochran's χ^2 based *Q* statistical test was performed in order to assess possible heterogeneity among the individual studies and, thus, to ensure that each group of studies was suitable for meta-analysis. The catmap was configured so that the random-effect model described by DerSimonian and Laird was applied in the presence of heterogeneity of the genetic effects ($p \leq 0.32$), while the fixed-effect model described by Mantel–Haenszel was applied in the absence of heterogeneity ($p > 0.32$). The significance of the pooled ORs and the heterogeneity of the group of ORs were assessed using a χ^2 test. The significance level for statistical tests was set at two-tailed $p < 0.05$.

3. Results

3.1. Genetic association analysis

Our study size of 1463 cases and 1795 controls had sufficient power (>0.98) to detect an effect at an odds ratio of 1.29 or larger, as described in the initial report, for each SNP (Zhao et al., 2007). The genotype and allele frequencies of five SNPs located in the *CHI3L1* gene and the flanking regions are summarized in Table 1. Significant differences in the genotype and allele frequency between patients and controls were observed in SNP4, which is located within the promoter region (genotype; $\chi^2 = 7.9$, $p = 0.019$, allele; $\chi^2 = 6.7$, $p = 0.009$). The significant association remained even after SNPSpD correction for multiple tests (the effective number of independent marker loci: 4.47; $p = 0.040$). The G-allele frequency at SNP4 was higher in patients (85.9%) than in controls (83.6%). There was no allelic or genotypic association with schizophrenia for the other four SNPs. Haplotype analysis showed several significant associations with schizophrenia (the most significant global $p < 0.001$, SNP3–SNP4–SNP5 and SNP3–SNP4–SNP5–rs2275351) (Table 2). This evidence for association remained positive after correction for multiple tests (10 independent global tests, the haplotypic association: $p = 0.0010$ after Bonferroni correction). The differences in detailed haplotype frequencies between cases and controls are provided in Supplementary Table 1. The LD relationships between markers are provided in Fig. 1. The LD pattern observed in our controls was nearly identical to that among our patients, the previously reported Chinese samples and JPT HapMap samples, but was different from those reported for the CEU and YRI HapMap samples. The moderate LD patterns observed between SNP5–rs2275351 were observed in both groups ($0.25 < r^2 \leq 0.50$).

3.2. Meta-analysis

We selected four studies using the Schizophrenia Research Forum and MEDLINE (Zhao et al., 2007; Yamada et al., 2008; Yang et al., 2008; Betcheva et al., 2009). The four studies and the present study (five case–control studies and two family-based studies) included 3005 patients, 3825 controls and 601 trios. The demography of the combined studies is shown in Table 3. No association between any SNP and schizophrenia was revealed in the overall population (Table 4). There was no heterogeneity among studies in SNP1 or SNP5 in the overall population. We found evidence of heterogeneity among studies of SNP3 ($p = 0.026$), SNP4 ($p = 0.00035$) and rs2275351 ($p = 0.048$). Heterogeneity in the meta-analysis refers to variation in study outcomes among studies. Thus, we analyzed two subdivided ethnic groups, Japanese populations

Table 1
Genotype and allele distributions for SNPs in the *CHI3L1* gene between patients with schizophrenia and controls.

Marker				SCZ			CON			Genotypic *p*-value (*df*=2)	SCZ	CON	Allelic *p*-value (*df*=1)	OR
SNP IDs[a]	Position[b]	M/m[c]	Gene	M/M	M/m	m/m	M/M	M/m	m/m		MAF			
SNP1	201426329	T/C	5′	0.57	0.36	0.08	0.58	0.36	0.06	0.14	0.26	0.24	0.13	1.09
SNP3	201422621	C/T	5′	0.45	0.44	0.11	0.46	0.42	0.12	0.73	0.33	0.33	0.71	1.02
SNP4	201422505	G/C	5′ UTR	0.74	0.23	0.02	0.70	0.28	0.03	**0.019**	0.14	0.16	**0.009**	0.83
SNP5	201419424	A/G	Exon 5	0.43	0.45	0.13	0.42	0.45	0.13	0.89	0.35	0.36	0.65	1.02
rs2275351	201416696	G/A	Intron 7	0.30	0.45	0.25	0.27	0.48	0.25	0.08	0.47	0.49	0.22	0.94

SCZ, patients with schizophrenia; CON, healthy controls; m, minor allele; M, major allele; MAF, minor allele frequency; OR, odds ratio. Significant *p* values are shown as bold face and underline.

[a] The db SNP IDs equivalent to the SNP IDs designed by Zhao et al. (2007) are the following: SNP1 (rs2364574), SNP3 (rs10399805), SNP4 (rs4950928), SNP5 (rs880633).

[b] db SNP build 129.

[c] The first shown alleles are major allele. All the alleles are represented according to the minus strand DNA sequence to make them comparable with the previous published data.

Table 2
Haplotype analysis of *CHI3L1* gene between patients and controls.

	Haplotypic global *p* values			
	Window level			
db SNP IDs[a]	2	3	4	5
rs2364574 (SNP1)				
	0.25			
rs10399805 (SNP3)		**0.018**		
	0.037		**0.0037**	
rs4950928 (SNP4)		**0.00010**		**0.0040**
	0.0038		**0.00010**	
rs880633 (SNP5)		**0.0017**		
	0.18			
rs2275351				

Haplotypes with frequencies <3% in each group are excluded. Significant *p* values are shown as bold face and underline.
[a] The db SNP IDs equivalent to the SNP IDs designed by Zhao et al. (2007) are shown in parentheses.

Table 3
Demography of the combined studies.

Authors	Ethnicities	Patients	Controls
Case–control studies			
Zhao et al. (2007)	Chinese	412	464
Yang et al. (2008)	Irish	375	812
Yamada et al. (2008)	Japanese	570	570
Betcheva et al. (2009)	Bulgarian	185	184
Ohi et al. (present study)	Japanese	1463	1795
Family-based studies			
Zhao et al. (2007)	Chinese	308 probands	
Yamada et al. (2008)	Chinese	293 probands	

(2033 patients and 2365 controls) and Chinese populations (412 patients, 464 controls and 601 trios), which were major groups across the five studies (Table 4). There was no heterogeneity among studies for these SNPs in Japanese and Chinese populations individually, expect for SNP4 in Chinese populations ($p=0.012$). We detected a significant association between SNP4 and schizophrenia in Japanese populations [$p=0.003$, OR = 0.84 (0.75–0.94)], while we detected a significant association between SNP3 and schizophrenia in Chinese populations [$p=0.003$, OR = 0.85 (0.76–0.95)]. These results remained significant even after Bonferroni correction (independent tests of the four SNPs, SNP4; corrected $p=0.012$, SNP3; corrected $p=0.012$).

4. Discussion

In this study, we found that SNP4 in the *CHI3L1* gene was associated with schizophrenia in a large Japanese population. Second, we performed a meta-analysis of the overall combined populations of several studies. In the meta-analysis, significant heterogeneity among studies was observed in SNP3 and SNP4. Because of the significant heterogeneity, we stratified the studies by ethnicity. We found that schizophrenia was associated with distinct SNPs in the *CHI3L1* gene in the Japanese and the Chinese populations.

We revealed a significant association of the G-allele of SNP4, which is located in the promoter region of the *CHI3L1* gene, with schizophrenia in a Japanese cohort (patients 85.9% vs. controls 83.6%). Our meta-analysis indicated a stronger association between SNP4 and schizophrenia in Japanese populations. Despite similar allele frequencies between cases and controls in the two Japanese cohorts, Yamada et al. (2008) reported no association between SNP4 and schizophrenia (patients 85.9% vs. controls 83.7%). This discrepancy might be attributed to the type II error for their small sample size (570 vs. 570) compared with our large sample size (1463 vs. 1795). In the meta-analysis of the overall combined population (Caucasian, Chinese and Japanese subjects), we found no association between these SNPs in the *CHI3L1* gene and schizophrenia. This result can be explained by the fact that the LD patterns in the HapMap data are different among each of these populations. For SNPs with heterogeneity among studies, we separately analyzed their association with schizophrenia in Japanese and Chinese populations. The meta-analyses showed that schizophrenia was associated with different variants (SNP3 and SNP4) in each population. Although the LD patterns between Asian populations were similar, the risk allele differed between Japanese and Chinese populations. It is unclear whether the difference resulted from subtle differences in LD patterns or allelic heterogeneity. It seems that an SNP might exist in this region that is more strongly associated with schizophrenia. This possibility could be addressed by re-sequencing or genotyping dense SNP mapping in this region and evaluating the association with schizophrenia.

It has been suggested that YKL-40 might be a potential biomarker for a cellular survival factor in an adverse microen-

Table 4
Meta-analysis of the genetic association studies for each SNP.

		Overall			Japanese			Chinese		
SNP ID	M/m	OR (95% CI)	$p(\chi)$	$p(Q)$	OR (95% CI)	$p(\chi)$	$p(Q)$	OR (95% CI)	$p(\chi)$	$p(Q)$
SNP1 (C)	T/C	(4)[a] 1.07 (0.99–1.15)	0.11[b]	0.74	(2)[a] 1.07 (0.97–1.18)	0.16[b]	0.53	(2)[a] 1.05 (0.93–1.19)	0.41[b]	0.37
SNP3 (T)	C/T	(6) 0.90 (0.80–1.01)	0.06	**0.026**	(2) 1.03 (0.94–1.12)	0.56[b]	0.79	(3) 0.85 (0.76–0.95)	**0.003**[b]	0.41
SNP4 (C)	G/C	(7) 1.03 (0.86–1.24)	0.72	**0.00035**	(2) 0.84 (0.75–0.94)	**0.003**[b]	0.90	(3) 1.29 (0.93–1.79)	0.13	**0.012**
SNP5 (G)	A/G	(4) 1.01 (0.94–1.08)	0.75[b]	0.80	(2) 1.00 (0.91–1.09)	0.98[b]	0.40	(2) 1.03(0.92–1.16)	0.58[b]	0.80
rs2275351 (A)	G/A	(2) 0.84 (0.65–1.09)	0.19	**0.048**	(1)–	–	–	(0)–	–	–

$p(\chi)$: chi-square test used determines the significance of the overall OR. Multiple testing corrections were not performed. Significant *p* values are shown as bold face and underline.
$p(Q)$: Cochran's *Q* test used to assess the heterogeneity. Random-effect model was applied in the presence of heterogeneity of the genetic effects ($p \leq 0.32$), while fixed-effect model was applied in the absence of heterogeneity ($p > 0.32$).
[a] The number of studies included in each meta-analysis is indicated in parentheses.
[b] This analysis was performed by fixed-effect model.

vironment because increased YKL-40 expression is found upon genotoxic and microenvironmental stress (i.e., hypoxia and ionizing radiation). It has been considered that a number of environmental stresses, such as fetal hypoxia and infection, in addition to genetic contributions, might induce susceptibility to schizophrenia (Palomo et al., 2004; Mittal et al., 2008). Patients with schizophrenia have shown increased levels of IL-6, IL-1RA and sIL-2R and a decrease in IL-2 (Potvin et al., 2008). YKL-40 is stimulated by IL-6 (Johansen et al., 2006), a multifunctional cytokine with varied system functions that plays a role in inflammatory processes and induces cell differentiation (Trikha et al., 2003). Cytokines play important roles in infection and inflammation and are crucial mediators of cross-talk between the brain and the immune system. Schizophrenia might be associated with an imbalance in inflammatory cytokines.

Elevated expression of the *CHI3L1* gene has been indicated in the hippocampus and prefrontal cortex in independent postmortem studies of patients who had schizophrenia (Chung et al., 2003; Arion et al., 2007). The G-allele at SNP4 has been associated with higher transcriptional activity according to a luciferase reporter assay and with higher *CHI3L1* mRNA levels in peripheral blood cells in patients with schizophrenia (Zhao et al., 2007). Interestingly, higher serum YKL-40 levels are involved in several inflammatory processes and tissue remodeling (Vind et al., 2003; Bergmann et al., 2005; Nordenbaek et al., 2005; Johansen, 2006; Kucur et al., 2007; Nojgaard et al., 2008). The G-allele at SNP4 occurred at a higher frequency in patients with asthma than in controls and was associated with higher serum YKL-40 levels (Ober et al., 2008). Our results suggest that the G-allele, which is enriched in patients with schizophrenia compared with controls, has a role in the etiology of schizophrenia. The risk *CHI3L1* genotype might be associated with serum YKL-40 levels in patients with schizophrenia. Further study of the possible association of *CHI3L1* genotype in patients with schizophrenia is required.

As schizophrenia is sensitive to environmental and psychological stresses (Leff, 1994; Howes et al., 2004), higher *CHI3L1* gene expression in patients with schizophrenia may be due to an excessive response to various stressors. SNP4, which is located within the promoter of the *CHI3L1* gene, might play a role in altering the expression and serum levels of YKL-40. In conclusion, we suggest that SNPs in the *CHI3L1* gene have ethnic heterogeneity and might contribute to the pathogenesis of schizophrenia in Asian populations. Further replication studies in other ethnic populations are required to confirm the possible relationship between *CHI3L1* and schizophrenia.

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Contributors

R. Hashimoto supervised the entire project, collected the data, wrote the manuscript, was critically involved in the design, analysis and interpretation of the data and was responsible for performing the literature review. K. Ohi was critically involved in the collection and analysis of the data, and contributed to the editing of the final manuscript and contributed intellectually to the interpretation of the data. Y. Yasuda, T. Yoshida, H. Takahashi, N. Iike, M. Iwase, K. Kamino, R. Ishii, H. Kazui, M. Fukumoto, H. Takamura, H. Yamamori, M. Azechi, K. Ikezawa, H. Tanimukai, S. Tagami, T. Morihara, M. Okochi, K. Yamada, S. Numata, M. Ikeda, T. Tanaka, T. Kudo, S. Ueno, T. Yoshikawa, T. Ohmori, N. Iwata, N. Ozaki and M. Takeda were heavily involved in the collection of the majority of the data and contributed intellectually to the interpretation of the data. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary table

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2009.12.002.

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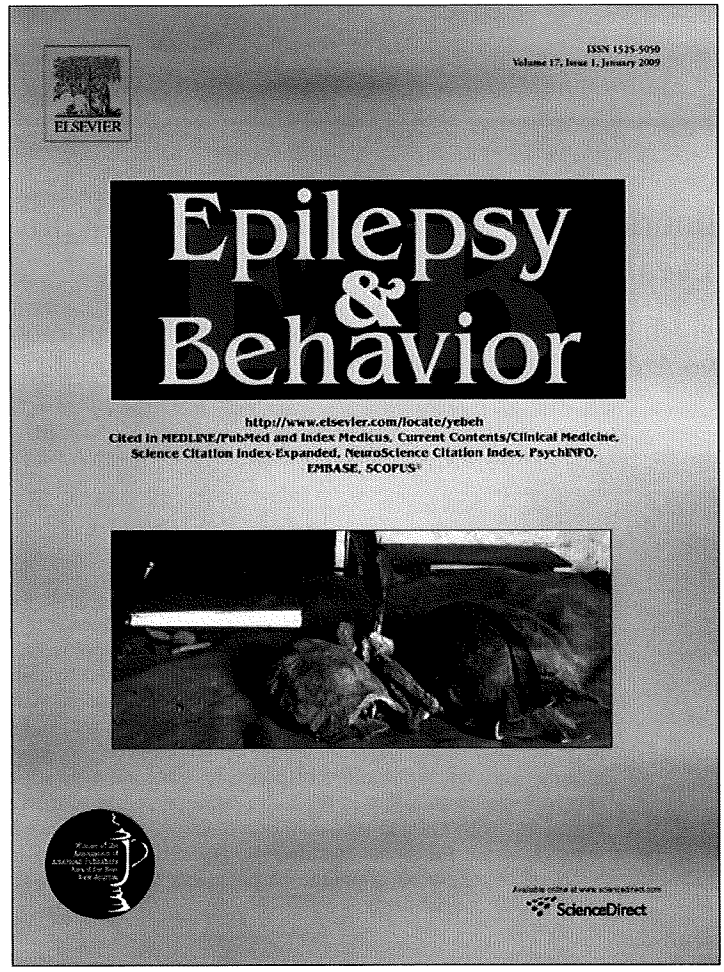

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Working memory abnormalities in chronic interictal epileptic psychosis and schizophrenia revealed by magnetoencephalography

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ABSTRACT

Working memory (WM) deficits are considered a core cognitive dysfunction in schizophrenia. To determine cognitive abnormalities in chronic interictal psychosis (CIP), and to assess whether these abnormalities are distinguishable from those seen in schizophrenia in terms of WM deficits, we used magnetoencephalography during a WM task performed by patients with CIP, nonpsychotic epilepsy, and schizophrenia and by healthy subjects. Multiple Source Beamformer and Brain-Voyager were used for analysis. In both patients with CIP and those with schizophrenia, we found dorsolateral prefrontal hyperactivation and left inferior temporal hypoactivation, as indicated by alpha event-related desynchronization and synchronization, respectively. Patients with schizophrenia also showed alpha2 event-related desynchronization in the mid-prefrontal cortex relative to healthy controls. Direct comparison of patients with CIP and schizophrenia rendered no difference in source-power changes. Our findings indicate similar functional cognitive abnormalities in CIP and schizophrenia in the prefrontal and left temporal cortex, which supports the possibility that these disorders share common underlying pathophysiological mechanisms.

1. Introduction

Psychosis is a common feature of epilepsy that may present during (*ictally*) or immediately after (*postictally*) seizures. If the psychotic episodes occur between seizures (*interictally*), implying their independence from seizure activity, they are classified as either *brief* or *chronic interictal psychosis* (CIP) [1–4]. Clinically, CIP closely resembles primary schizophrenia with a typical presentation as a paranoid hallucinatory syndrome, which is why it is also referred to as *schizophrenia-like psychosis of epilepsy* (SLPE). Despite the similarities, there is an ongoing debate as to whether CIP and schizophrenia share common pathophysiology. Compared with the latter, CIP distinguishes itself by a relative absence of negative symptoms and better premorbid as well as long-term functioning [1,5–7]. However, similar personality deterioration has been noted in CIP and schizophrenia [8]. With some certainty, the ictal psychotic state is a direct effect of epileptic discharges, particularly when limbic or paralimbic structures are involved, whereas postictal psychosis is likely associated with seizure-induced neurochemical changes in the brain. Entirely unclear is how CIP relates to epilepsy—whether it is a result of former seizures or possibly merely coincidental [1,6]. Although several factors such as early onset of epilepsy [8,9], seizure exacerbation, and small hamartomas and dysplasias [1] have been linked to CIP, to date, the etiology and pathophysiology of CIP are poorly understood.

Despite extensive efforts after the initial description of CIP made by Slater and colleagues in 1963, CIP phenomenology is still being debated, and a distinct clinical entity separating it from schizophrenia is not yet identified [6,8,10–16]. Perez and Trimble found a relationship between schizophrenia-like symptoms and temporal lobe epilepsy [10]. However, clinical–epidemiological studies have reported substantial differences between CIP and primary schizophrenia, including more delusions of reference and fewer catatonic features [11], as well as higher remission rates with lower doses of antipsychotic drugs, in patients with CIP [17]. Thus, these studies support the concept that CIP is a distinct nosological entity. Yet, reports of almost identical neuropsychological profiles for CIP and primary schizophrenia listing impairment in attention [13], memory, and executive function [13,16] speak against this notion. The aforementioned reports implicate not only temporal lobe deficits, but also generalized cognitive abnormalities regardless of whether the psychosis is associated with CIP or schizophrenia. Generally, it is quite difficult to distinguish the

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two conditions on grounds of epidemiology, phenomenology, and neuropsychology alone. Neuroimaging studies have attempted to resolve this dilemma. First successes have been reported in approaches using MRI [19] and MRI spectroscopy [12], where left or bilateral temporal volume reductions, particularly in the amygdala and hippocampus, have been found to be specific to patients with CIP. There is also evidence from advanced MRI techniques, such as diffusion tensor imaging [19] and magnetization transfer imaging [20], visualizing changes in the temporal or frontal temporal lobe such as subtle structural abnormalities in the white and gray matter of patients with CIP. Prominent cortical pathology of this type, specifically when it affects the temporal lobe and when amygdala volume reduction is pronounced, is also seen in schizophrenia, which upholds the idea that these disorders share similar pathophysiological mechanisms. Other MRI work looking at volumetric and morphometric differences between patients with interictal psychosis and patients with nonpsychotic epilepsy has demonstrated amygdala enlargement [21] and lack of cortical gray matter abnormalities [22] in CIP, which has never been reported in schizophrenia.

To date, most MRI work has focused on structural abnormalities in CIP, with only a few functional neuroimaging studies directly comparing psychotic and nonpsychotic patients with epilepsy to subjects with schizophrenia. These studies, using PET or SPECT, report reduced cerebral blood flow [23,24] and decreased metabolism [25] in patients with interictal psychosis, predominantly in left temporal and frontal regions during rest [23] and during verbal fluency task performance [24], which speaks for frontotemporal cortex involvement in CIP. Neuroimaging studies have also complemented neuropathology findings in both schizophrenia and CIP, including ventricular enlargement and heterotopias, which may be the result of neuronal migration defects, hinting toward common neurodevelopmental mechanisms [1,6]. Furthermore, primary schizophrenia and CIP share not only key diagnostic symptoms such as hallucinations, delusions, and disorganized thought processes, but they also converge on impairments in cognitive functions [13,16,26]. In particular, deficits in working memory (WM) are considered a core cognitive abnormality in schizophrenia, which underlies diverse neuropsychological impairments stemming from a variety of neural systems [27].

There is a growing body of functional neuroimaging evidence on WM deficiency in schizophrenia. Among others, it has been demonstrated that certain cortical areas, especially the dorsolateral prefrontal cortex (DLPFC), are implicated in both WM deficits and the pathophysiology of schizophrenia [28]. Yet, only little work has been done to examine a possible connection between cognitive abnormalities in CIP and schizophrenia that may help elucidate the relationship between the two. Toward that end, brain oscillatory activity may be of particular interest, which, until recently, was focused mostly on schizophrenia resting state electroencephalography (EEG) [29]. However, several studies have demonstrated that task-induced changes in brain oscillatory activity in different frequency bands provide important clues to underlying cognitive processes [30,31]. Especially with respect to memory and integrative function, induced oscillatory activity may be the key to understanding functional communication in the brain [32]. Oscillations in the theta frequency band, for instance, consistently correlate with WM demands [33,34], and the specific enhancement in medial prefrontal areas known as frontal medial theta activity is closely associated with focused attention, which is critical to WM and other types of cognitive tasks [35]. Changes in the alpha band have also been implicated in attention and WM function [34,36]. With the advent of advanced time–frequency analysis, recent neurophysiological studies have begun to examine task-related oscillatory activity in patients with schizophrenia using EEG or magnetoencephalography (MEG). Abnormal oscillations have been associated with cognitive dysfunctions and several symptoms of schizophrenia [29,37,38], but to date, the same approach has not yet been exploited to examine cognitive dysfunction in CIP.

Unlike other neuroimaging modalities such as fMRI, SPECT, and PET, which rely on the hemodynamic or metabolic changes that may occur in response to neural activity, MEG is a neurophysiological technique that measures neural activity directly and noninvasively, with high temporal and spatial resolution [39]. Co-registration of MEG data to structural MRI also allows for functional mapping of the cortex based on power changes in oscillatory activity within specific frequency bands during cognitive task performance [31]. Using a variation of this methodology, we previously mapped hallucination-induced cortical activation in schizophrenia [40] and delusions in brief interictal epileptic psychosis [3]. Magnetoencephalography thus appears to be a useful tool in the localization of cortical regions associated with WM dysfunction while at the same time providing information on the underlying neural activity.

In the work described here, we used MEG to determine functional cognitive abnormalities in CIP and schizophrenia, and to assess whether these disorders can be distinguishable in terms of WM deficits. By focusing on source-power changes in brain oscillatory activity during performance of a visual-object WM task, we contrasted patients with CIP with those with epilepsy without psychosis and with those presenting with schizophrenia. Patients with schizophrenia were compared with healthy controls. Special attention was given to the potentially confounding effect of medication. The working hypothesis was that patients with CIP and those with schizophrenia have similar patterns of abnormal oscillatory activity related to WM dysfunction based on converging clinical and neuropsychological features.

2. Methods

2.1. Subjects

Twelve patients with CIP, 14 patients with nonpsychotic epilepsy (nPE), 14 patients with schizophrenia, and 14 healthy subjects participated in this study. Patients were recruited from inpatient and outpatient facilities of Osaka University Hospital. The patients in the epilepsy groups were ascertained from a database used in a previous study [41], and consisted of patients with secondary or cryptogenic focal epilepsy matched for age, gender, side of seizure foci, and type of epilepsy (i.e., temporal or frontal lobe epilepsy). Epilepsy classification followed the standards set by the International League Against Epilepsy (ILAE, 1989). Patient's ages ranged from 18 to 50 years. Epilepsy diagnosis and seizure focus localization were determined by neurologists and neuropsychiatrists on the basis of (1) seizure semiology, (2) findings from ictal or interictal scalp EEG, and (3) MEG dipole fitting or co-registration of spatially filtered MEG data [42,43] with MRI results. Epilepsy-related information such as estimated seizure frequency in the previous year, age at seizure onset, and illness duration was obtained from patient and family interviews as well as from chart reviews. All patients with CIP had chronic psychosis that developed after the onset of epilepsy. Patients with schizophrenia were ascertained from a schizophrenia research database at Osaka University Hospital [44]. Patients with schizophrenia and CIP were psychiatrically diagnosed by two or more independent psychiatrists on the basis of a structured clinical–psychiatric interview using medical charts and clinical criteria as listed in the fourth edition of the *Diagnostic and Statistical Manual* (DSM-IV; American Psychiatric Association, 1994). All groups were matched for age and gender. Healthy controls were recruited through local advertisement. A prerequisite for admission was a history—personal as well as

first- and second-degree relatives—clear of neurological or psychiatric disease. All healthy controls were matched to the schizophrenia group in age, gender, and premorbid IQ. Screening for psychiatric disease was performed with the nonpatient edition of the modified Structured Clinical Interview for the *Diagnostic and Statistical Manual*, Fourth Edition, Axis I disorders (SCID-I/NP) [45]. Patients with postictal or brief interictal psychotic episodes and psychoses in the context of status epilepticus or drug toxicity/withdrawal were excluded, as were patients with organic lesions other than intrinsically epileptogenic cortical dysplasia ($N = 2$) or hippocampal sclerosis ($N = 2$). Other exclusion criteria were: age greater than 55, history of drug or alcohol abuse, and IQ score below 70, as indicated by the Wechsler Adult Intelligence Scale—Revised (WAIS-R). Most patients with epilepsy were on carbamazepine or valproic acid monotherapy, with some taking phenytoin as adjunctive treatment. Patients with psychosis were on regimens involving neuroleptic drugs—mostly atypical antipsychotics—such as risperidone, olanzapine, and aripiprazole. None of the patients were taking sedatives or antidepressants. None of the healthy controls underwent any pharmacological treatment that could have affected EEG or MEG activity at the time of the study. All subjects were right-handed as assessed by the Edinburgh Handedness Inventory [46]. Written informed consent was obtained from all subjects prior to the experiments, and ethical approval was obtained from the Ethics Committee of Osaka University Hospital.

2.2. Clinical and neuropsychological assessment

The Brief Psychiatric Rating Scale (BPRS) was used to assess psychopathology in patients with CIP on the day of the MEG recordings. Positive and negative symptom scores were obtained using the four-factor model of the BPRS [47]. Patients with schizophrenia were assessed with the Positive and Negative Syndrome Scale (PANSS) [48] during the previous week, as part of routine diagnosis and treatment assessment. The premorbid (preschizophrenia) IQ in patients with schizophrenia and healthy controls was estimated using the Japanese version of the National Adult Reading Test (JART) [49]. Full IQ was obtained for all patients and healthy controls using the Wechsler Adult Intelligence Scale—Revised (WAIS-R) to exclude subjects with mental retardation.

2.3. Experimental task

The patients performed a modified version of Sternberg's memory recognition task [50] which emphasizes a visual-object WM task. During the MEG recordings, 3 seconds after a visual "START" cue, a set of five digits (*memory set*) were randomly presented on a screen for 2 seconds, and the patients were asked to memorize them (*memory encoding phase*). After a 5-second *memory retention period*, a series of three single probe digits of 1-second duration and a 2-second interstimulus interval were displayed, and the patients were asked to push a button held in their right hand if the probe was included in the previous set or a button in their left hand if it was not (*memory recognition phase*) (Fig. 1). For each trial, a cue "+" symbol was displayed before the memory set, during the retention period, during the interstimulus interval, and during the 8-second intertrial intervals. The total number of trials was 20, each lasting 27 seconds, resulting in a total duration for the task of approximately 9 minutes per subject. Before the experiment, all subjects were given complete task instructions; practice trials were performed to ensure familiarity with the procedure. The subject's recall percentage was determined, and those with less than 75% correct answers were excluded from the analyses [50].

2.4. MEG data acquisition

Neuromagnetic data were recorded at 625 Hz with a bandwidth of DC-100 Hz using a 64-channel MEG system equipped with a whole-head array of first-order radial SQUID gradiometers (NeuroSQUID Model 100, CTF Systems, Inc., Canada). During the recordings, the subjects were sitting on a comfortable chair in a magnetically shielded room with the head positioned in the helmet-shaped Dewar. The localization of the subject's head relative to the sensor array was measured with three coils affixed to the nasion and preauricular points.

2.5. Data analysis

2.5.1. Time–frequency analysis

All data analysis was performed offline. MEG channels and trials with signal variations larger than 3 pT/cm were considered as

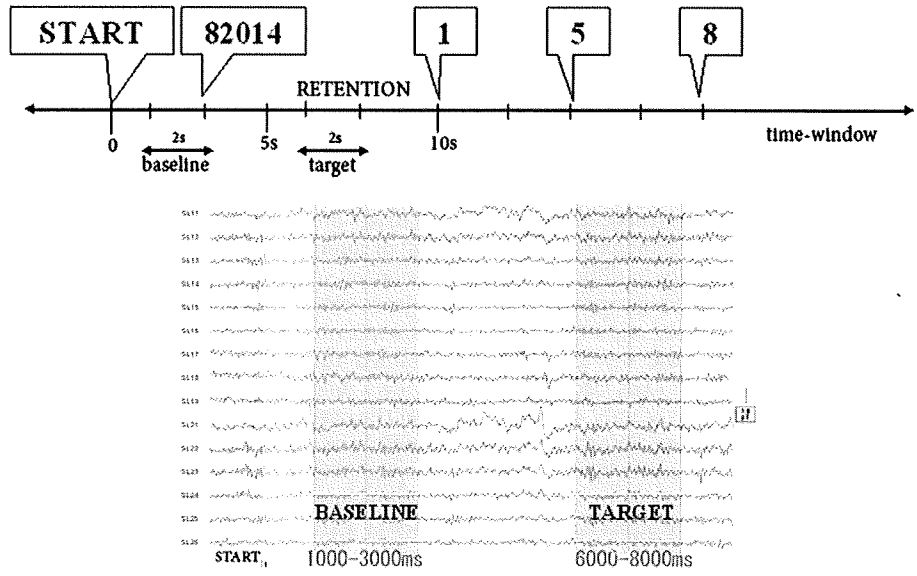

Fig. 1. Schematic representation of an example trial used during the modified version of Sternberg's paradigm in our study. Each trial included the presentation of a string of five digits (memory set) for 2 seconds (3 seconds after the "START" cue); a 5-second retention interval, in which the subjects were asked to memorize the digits; and the sequential presentation of three single probe digits of 1-second duration. The total number of trials was 20. At the bottom is an example of MEG waveforms during a trial. The shadowed areas indicate the baseline (time window: 1–3 seconds after the "START" cue) and target (time window: 6–8 seconds after the "START" cue) intervals.