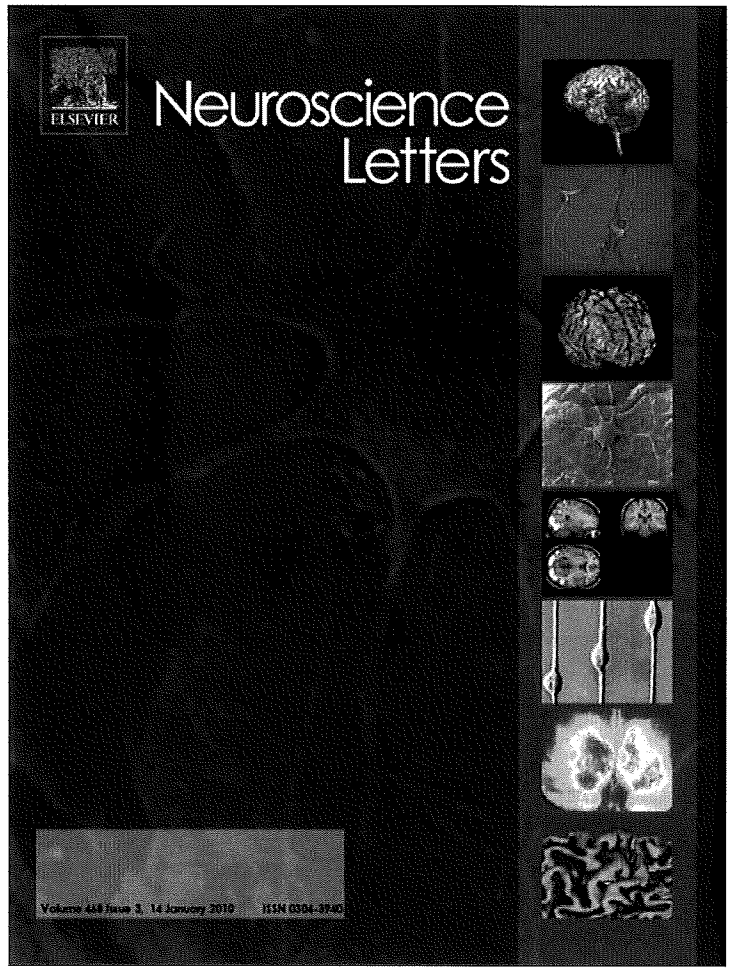

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Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder

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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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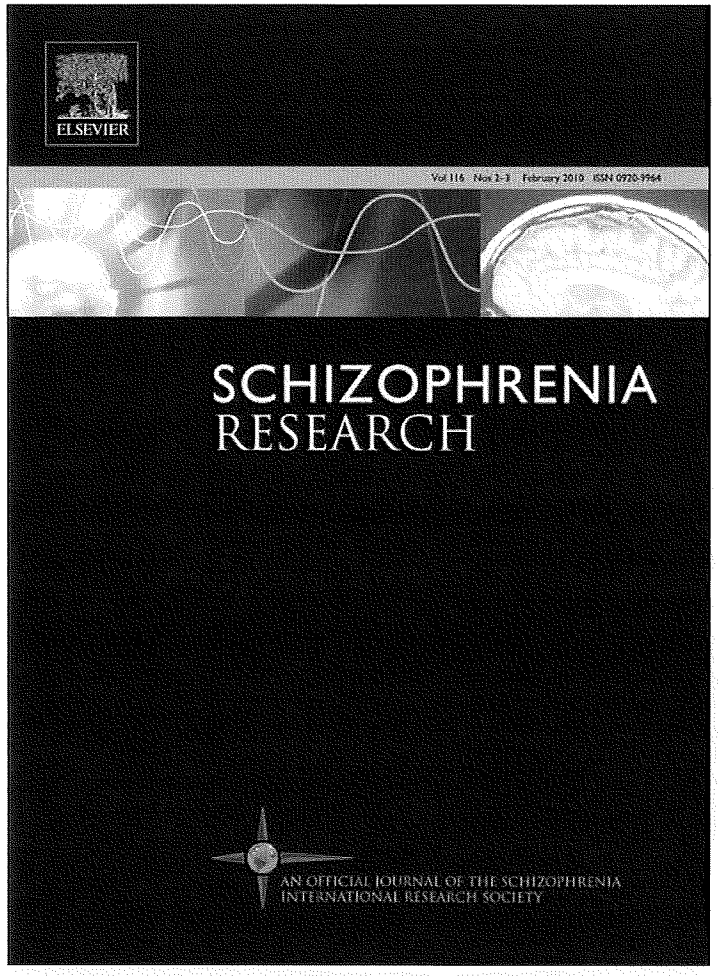

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The *chitinase 3-like 1* gene and schizophrenia: Evidence from a multi-center case–control study and meta-analysis

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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 *CHI3LI* 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

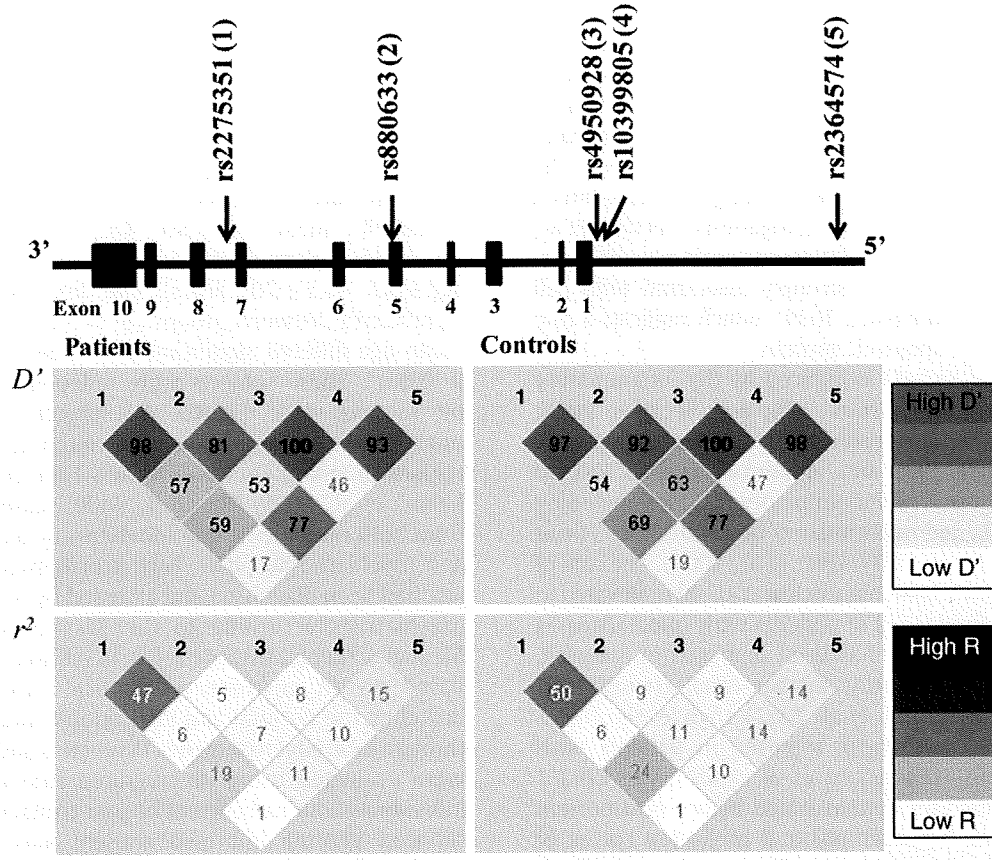

Fig. 1. Genomic structure of *CHI3L1*, including locations of the five SNPs studied, and linkage disequilibrium of these five SNPs in the patient and control groups. Based on an entry in the Entrez Gene database (National Center for Biotechnology Information), the genomic structure of *CHI3L1* is shown 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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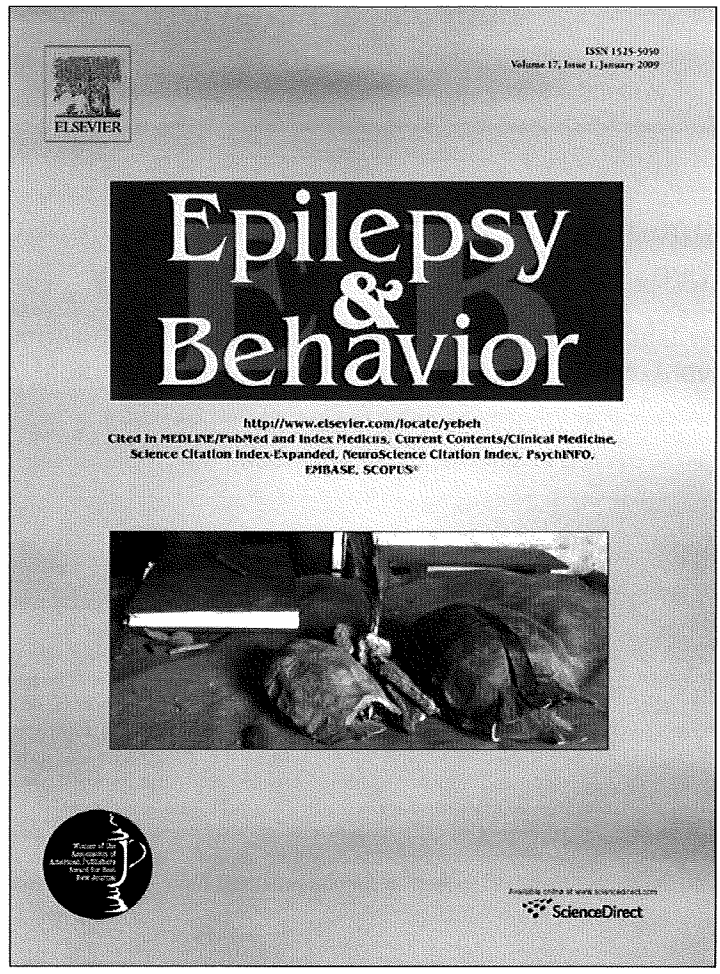

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Working memory abnormalities in chronic interictal epileptic psychosis and schizophrenia revealed by magnetoencephalography

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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

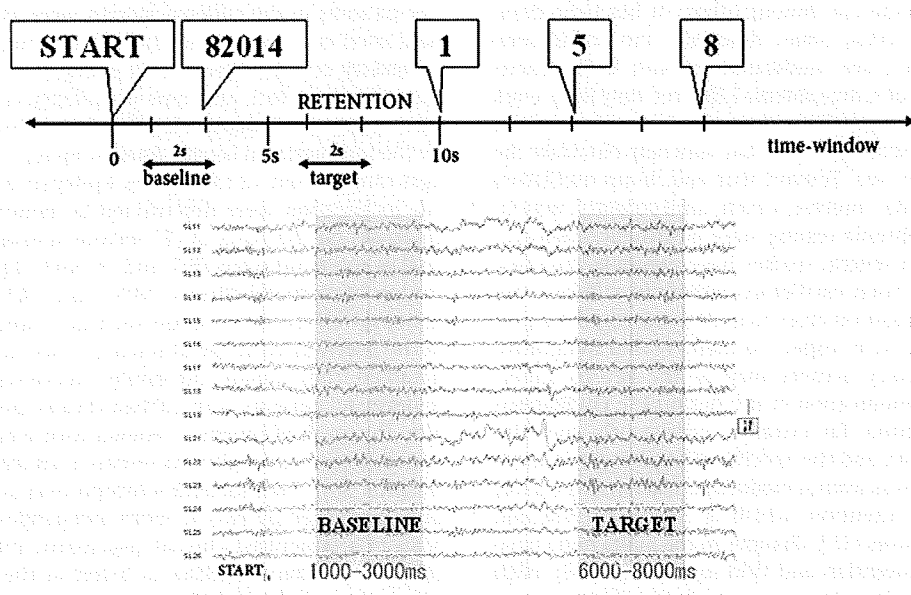

Fig. 1. Schematic representation of an example trial used during the modified version of Sternberg's paradigm in our study. Each trial included the presentation of a string of five digits (memory set) for 2 seconds (3 seconds after the "START" cue); a 5-second retention interval, in which the subjects 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.

including artifacts and were excluded from further analysis [51]. Source imaging of MEG data in the time–frequency domain was performed using the multiple source beamformer (MSBF) implemented in Brain Electrical Source Analysis (BESA) software (Fig. 2). The MSBF is a modified version of the linearly constrained minimum variance vector beamformer in the time–frequency domain [52]. As an adaptive beamformer, the MSBF applies a spatial filter specific for each brain voxel that is fully sensitive to activity from the target voxel, while being as insensitive as possible to activity from other brain regions, thus suppressing interference from unwanted signals. We analyzed frequencies between 1 and 60 Hz in 2-Hz steps; latencies were sampled in steps of 25 ms. The BESA beamformer applied complex demodulation to transform time-domain MEG data into time–frequency data. This provided information on the envelope amplitude and the phase of a specified frequency band as a function of time [51,53]. The complex demodulation consisted of a multiplication of the time-domain signal by a complex periodic potential function with a frequency equal to the frequency analyzed and an additional low-pass filter. This low-pass filter was a Gaussian-shape finite impulse response filter in the time domain, which is related to the envelope of the moving window in wavelet analysis. In the resulting complex signal, its magnitude corresponded to half the envelope amplitude and its phase to the compound phase of the filtered frequency band. To obtain power values, the time-series MEG data were squared and averaged across all 20 trials. Time–frequency representations of changes in power normalized to baseline for each MEG sensor were obtained from each subject. An increase in power at a specific frequency compared with the mean power of the baseline period for that frequency was defined as event-related synchronization (ERS), whereas a decrease in power was deemed event-related desynchronization (ERD). Power changes displayed on a spectrogram are shown as percentages of ERS/ERD with color-coded intensities; the *x* axis and *y* axis relay the time relative to the "START" cue and the frequency, respectively (Fig. 2a). For each trial, a 2-second interval before the memory set (time window: 1–3 seconds after the "START" cue) was deemed baseline, and a 2-second interval after the memory set (time window: 6–8 seconds after the "START" cue) was deemed target interval (memory retention period) (Fig. 1). Source-power changes (ERD/ERS) were measured in five frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (30–60 Hz). The alpha band was additionally subdivided into alpha1 (8–10 Hz) and alpha2 (10–13 Hz).

2.5.2. Statistical group analysis

To image induced oscillatory activity, BESA (www.besa.de) computed the complex cross-spectral density matrices (the time–

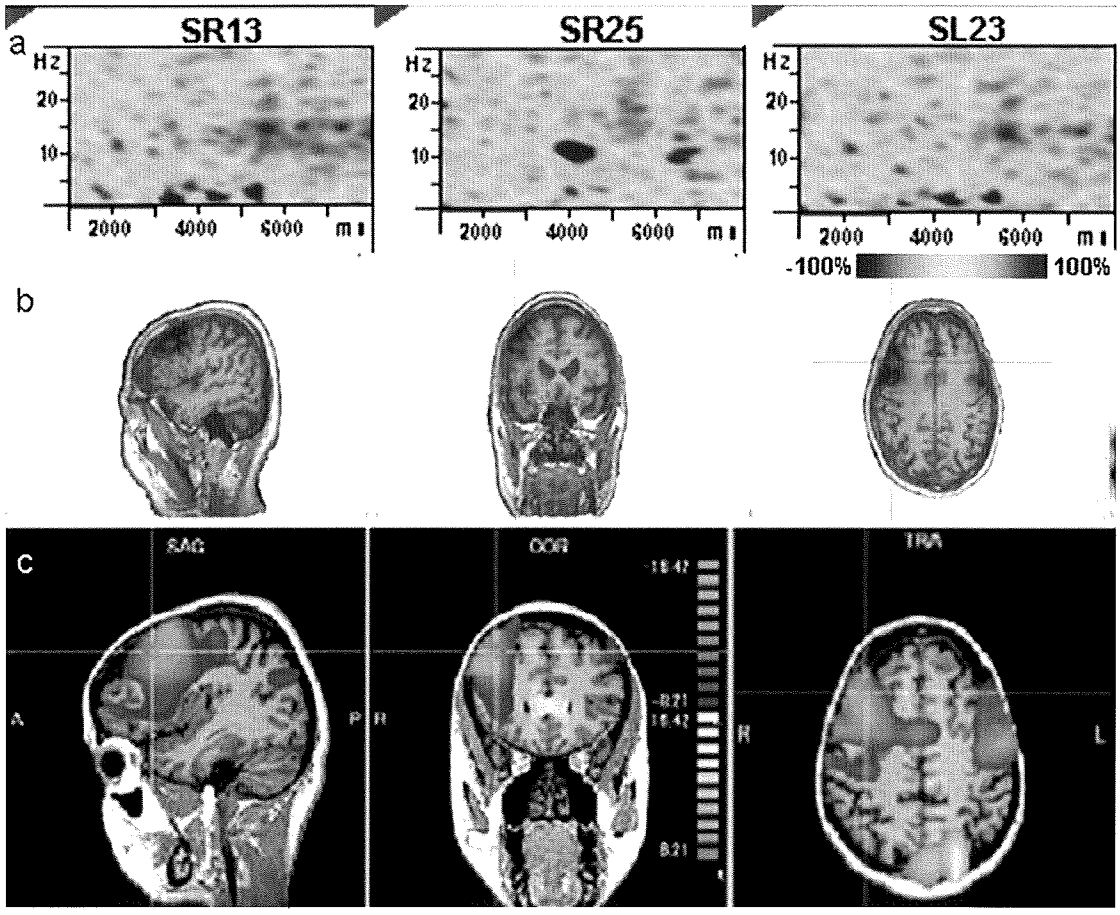

Fig. 2. Schematic representation of the time–frequency analysis. (a) Time–frequency plots for MEG channels showing power changes (averaged across all trials) in the alpha band (8–13 Hz) in a representative patient with chronic interictal psychosis. In the spectrogram, the *x* axis denotes the time relative to the "START" cue (ms), and the *y* axis denotes the frequency of oscillatory activity (Hz). Sustained event-related desynchronization (ERD) is observed in the 6- to 8-second time window (target interval) in channels overlying the right (SR13) and left (SL23) dorsolateral prefrontal cortex (DLPFC). A strong event-related synchronization (ERS) is observed in the target interval in the SR25 channel, which overlies the midline parietal region. (b) Multiple source beamformer (MSBF) analysis using BESA software. Color-coded maps show the magnitude change in the target interval relative to baseline as a percentage for the analyzed frequency band (alpha). (c) Brain Voyager image of source-power changes. The resulting beamformer three-dimensional images are exported to Brain Voyager QX and superimposed onto a T1-weighted brain template for group statistics. The color bars represent the percentages of decrease (blue/green, ERD) and increase (red/yellow, ERS) in oscillatory activity power changes. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

frequency equivalent of the data covariance matrix) for the target and baseline intervals from single-trial data for each frequency band of interest. Color-coded maps were obtained displaying q values as a measure of the magnitude change in the target interval relative to the baseline in percent (Fig. 2b). The beamformer three-dimensional images superimposed onto the BESA standard anatomical magnetic resonance image reveal locations of the generators of the induced oscillatory activity in the specified frequency. These images were exported for further statistical analysis to the Brain Voyager QX software package (Brain Innovation, Maastricht, The Netherlands) to assess between-group differences in source-power changes for each frequency band (Fig. 2c). BrainVoyager QX, originally developed for fMRI analysis [54], was used for group analysis of MEG data [55]. The subject's three-dimensional images of source-power changes were superimposed onto a Talairach-transformed Montreal Neurological Institute (MNI) T1-weighted brain template in Brain Voyager, and the anatomical T1 coordinates in the statistical maps were transformed into Talairach coordinates to identify brain regions with significant between-group differences in source-power changes. Between-group comparisons of brain activation patterns, as indicated by ERD/ERS values in a given frequency band, were based on t-test statistics (two-tailed, unpaired) in BrainVoyager QX. For correlation of neural activity in psychotic patients with external clinical variables, particularly medication dosage, the coordinates at the voxel with t_{maxima} (peak ERD/ERS value) at locations with significant power changes in the statistical maps were obtained. Then, the value at these coordinates in each individual's three-dimensional functional image for each one of the frequency bands was determined for analysis. To minimize the risk of false-positive findings, all activation foci were set to a minimum cluster size of 20 voxels [56], and statistical results with an uncorrected P value < 0.001 were considered significant.

To test our hypothesis, we compared source-power changes in oscillatory activity across three groups: (1) patients with CIP versus patients with nPE, (2) patients with schizophrenia versus healthy controls, and (3) patients with CIP versus those with schizophrenia. Analysis of groups 1 and 2 focused on patterns of WM dysfunction, whereas analysis of group 3 tested whether CIP and schizophrenia differed in functional cognitive abnormalities. Correlation analyses were carried out using Pearson's correlation coefficient to examine the association of medication dose with peak ERD/ERS values at sources with significant power changes in a given frequency band in patients with CIP and schizophrenia. The χ^2 test was performed for independence of group and gender. Demographic and clinical variables were analyzed using an unpaired two-tailed t test or ANOVA, with the significance level set at $P < 0.05$. Results are expressed as means ± SD. These statistical analyses were carried out using SPSS software (SPSS, Inc., Chicago, IL, USA).

3. Results

The clinical and demographic information of patients with CIP, nPE, and schizophrenia and of healthy controls is given in Table 1. There were no significant differences between the epilepsy groups (CIP vs nPE) with respect to age at epilepsy onset, duration, or seizure frequency. The majority of these patients had temporal lobe epilepsy (left $N = 10$, right $N = 10$, bilateral $N = 2$); only four patients (two in each group) had a diagnosis of frontal epilepsy. The patients with epilepsy were taking mainly carbamazepine or valproic acid. Approximately two-thirds of these patients ($N = 16$, 61.5%) were on monotherapy (CIP: $N = 5$, 41.7%; nPE: $N = 11$, 78.6%), and two patients in the CIP group were drug free. There were no between-group differences in plasma levels of these drugs. All patients with CIP had chronic interictal psychosis with onset 14.8 ± 8.6 years after the epilepsy. Comparison between the psychotic groups (CIP vs schizophrenia) revealed no significant difference in age at psychosis onset, although patients with schizophrenia presented with slightly longer psychosis duration. The analysis of chlorpromazine equivalents showed that patients with schizophrenia were taking higher doses of antipsychotic drugs than were patients with CIP. Although there was no difference in education across groups, post hoc ANOVA with Bonferroni correction revealed that patients with CIP, as well as those with schizophrenia, had lower IQs than healthy controls ($p < 0.005$ and 0.003, respectively); no significant differences in IQ were found between the epilepsy groups (CIP vs nPE) or the psychosis groups (CIP vs schizophrenia). Patients with schizophrenia and healthy controls did not differ in premorbid (preschizophrenia) IQ ($t[26] = -0.07$, $P = 0.94$), as assessed with JART.

3.1. Behavioral data

All subjects achieved an accuracy rate of 75% or higher on the WM task. Using ANOVA we found a significant difference in the correct-response percentage across groups ($F = 5.35$, $P = 0.003$), with patients with CIP performing worse (90.5 ± 7.1%) than those with nPE (96.7 ± 1.7%, $P = 0.013$) and healthy controls (97.4 ± 2.8%, $P = 0.004$). However, the psychosis groups did not differ in correct-response percentage (schizophrenia: 94.1 ± 6.0%, $P = 0.367$) (Fig. 3). Reaction times also significantly differed across

Table 1
Demographic and clinical data[a].

Characteristic	nPE ($N = 14$)	CIP ($N = 12$)	SCZ ($N = 14$)	HC ($N = 14$)	P
Age	33.6 ± 12.2	34.8 ± 12.7	34.9 ± 10.2	34.2 ± 11.3	0.99
Gender (F/M)	10/4	8/4	9/5	9/5	—
Education, years	14.4 ± 2.1	13.5 ± 2.4	14.2 ± 1.8	15.1 ± 1.8	0.23
WAIS-R IQ	99.1 ± 13.4	94.2 ± 7.0[b]	94.2 ± 11.8[b]	109.6 ± 10.5	0.002
Premorbid IQ			100.6 ± 9.4	101.2 ± 24.8	0.94
Epilepsy onset, years	20.4 ± 11.6	14.8 ± 12.2			0.25
Epilepsy duration, years	13.1 ± 10.1	18.5 ± 9.8			0.19
Seizure frequency/year	6.4 ± 10.2	11.1 ± 18.6			0.42
Epilepsy type (TLE/FLE)	12/2	10/2			0.88
CBZ plasma levels, μg/mL	5.7 ± .9.1	6.9 ± 3.0			0.37
VPA plasma levels, μg/mL	61.5 ± 35.9	44.0 ± 24.9			0.28
CPZ equivalents		341.7 ± 387.0	1021.9 ± 806.4		0.015
Psychosis onset, years		29.6 ± 14.9	20.3 ± 4.5		0.062
Psychosis duration, years		5.2 ± 4.3	14.6 ± 11.6		0.013

[a] Data are means ± SD unless otherwise noted. nPE, nonpsychotic epilepsy; CIP, chronic interictal psychosis; SCZ, schizophrenia; HC, healthy controls, WAIS-R, Wechsler Adult Intelligence Scale–Revised; TLE, temporal lobe epilepsy; FLE, frontal lobe epilepsy; CBZ, carbamazepine; VPA, valproic acid; CPZ, chlorpromazine.
[b] Significant difference relative to HC (post hoc ANOVA).

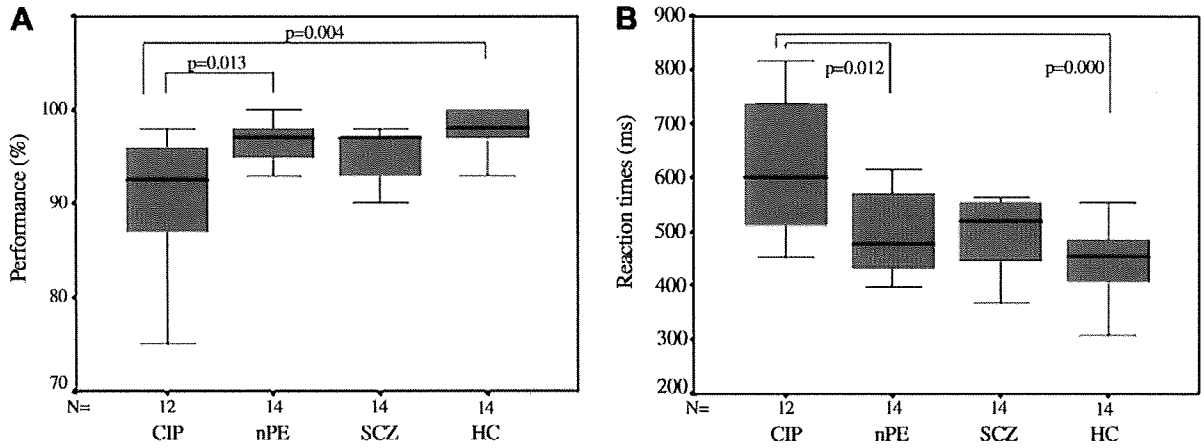

Fig. 3. Behavioral performance data as box plots. (A) Percentage of accurate response. (B) Reaction times. The boxes represent interquartile ranges. The whiskers show the highest and lowest values, excluding outliers. The lines across the boxes indicate medians. CIP, chronic interictal psychosis; nPE, nonpsychotic epilepsy; SCZ, schizophrenia; HC, healthy controls.

groups ($F = 7.15$, $P < 0.001$). The psychosis groups did not differ in reaction times (CIP: 619 ± 134 ms; schizophrenia: 519 ± 103 ms, $P = 0.066$), but patients with CIP had significantly longer reaction times compared with patients with nPE (495 ± 75 ms, $P = 0.012$) and healthy controls (448 ± 64, $P < 0.001$), as indicated by post hoc ANOVA with Bonferroni correction.

3.2. Power change analysis

We averaged the ERD/ERS percentages in different frequency bands and superimposed the data on a standard brain image (Fig. 4). This revealed that pronounced source-power changes exceeding 10% occurred only in alpha and theta frequency bands,

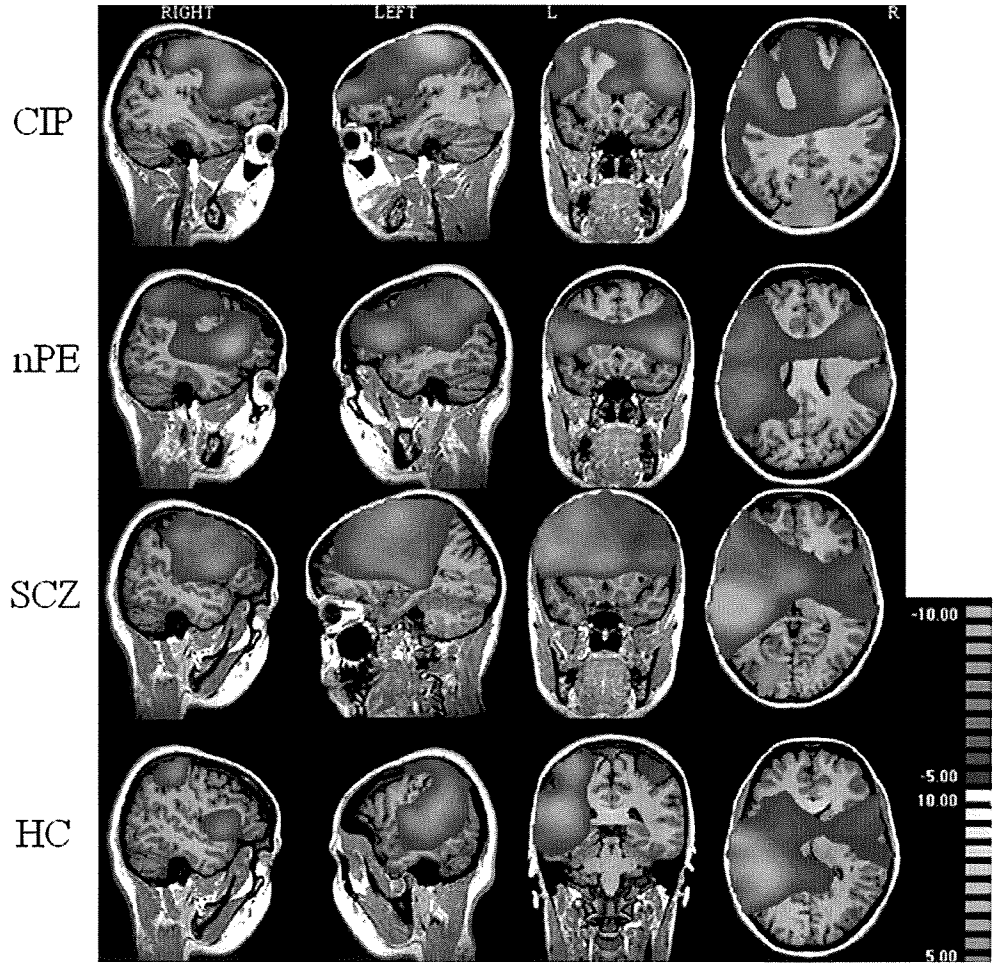

Fig. 4. Averaged alpha event-related desynchronization (ERD) and synchronization (ERS) greater than 5% across all groups using Brain Voyager. The color bars represent the percentages of decrease (blue/green, ERD) and increase (red/yellow, ERS) in oscillatory activity power changes. L, left; R, right; CIP, chronic interictal psychosis; nPE, nonpsychotic epilepsy; SCZ, schizophrenia; HC, healthy controls. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

particularly as ERD during WM retention. The alpha power was reduced bilaterally by 11.8% in the central-parietal regions and 11.5% in the right DLPFC, particularly over the superior frontal gyrus in patients with CIP. In patients with nPE, peak-averaged alpha ERD values >10% were only observed bilaterally in the DLPFC over the inferior frontal cortex (right: 10.7%, left: 10.3%) and bilaterally in the parietotemporal cortex (right: 10.2%; left: 10.8%).

In the theta frequency band, peak-averaged ERD > 10% was observed in patients with CIP over the right medial prefrontal and right parietal areas; this was not seen in patients with nPE (peak-averaged theta ERD 8.4%). In both patients with schizophrenia and healthy controls, peak-averaged alpha ERD values >10% were noted in central-parietal regions (schizophrenia: 15%; controls: 13%). In addition, there was pronounced ERD in the DLPFC (peak value: 11%) and in medial areas (peak value: 10.8%) in patients with schizophrenia and in the posterior temporal cortex in the controls (12.5%). Peak-averaged alpha ERD in the DLPFC was smaller than 8.5% in control subjects. Pronounced theta ERD was observed in parietal regions, predominantly on the left side (peak value: 12%) in patients with schizophrenia. At a threshold of 5% of power changes, alpha ERS was observed in patients with CIP and schizophrenia, but not in patients with nPE and healthy controls. This increase in alpha power occurred over posterior regions, particularly in medial parietal-occipital (peak value: 8.4%) and left posterior temporal (peak value: 7.7%) cortices in patients with CIP, and in the left temporal–occipital cortex (peak value: 6.7%) in patients with schizophrenia.

3.3. Between-group differences in source-power changes

The analysis of source-power changes in brain oscillatory activity during the retention period of the WM task between patients with CIP and those with nPE indicated significant differences in alpha frequency band: patients with CIP showed ERD in the right DLPFC and ERS in medial parietal areas, specifically in the right precuneus and left posterior parietal cortex, as well as in the left temporal lobe, namely, the inferior-posterior temporal cortex (Fig. 5). The findings in peak alpha ERD/ERS values at t_{maxima} of these sources are summarized in Table 2. When the alpha band was divided into alpha1 (8–10 Hz) and alpha2 (10–13 Hz), we observed findings similar to those for the full alpha band with respect to power changes in the DLPFC and midparietal areas. In particular, patients with CIP and nPE differed significantly in alpha1 ERS in parietal areas, including the precuneus and the posterior parietal cortex (peak ERS value: CIP 5.4 ± 4.3%; nPE −6.3 ± 4.4; $t = 6.80$, $P < 0.001$), and in alpha2 ERD in the DLPFC (peak ERD value: CIP −7.8 ± 3.5%; nPE −0.68 ± 4.2%; $t = -4.2$, $P < 0.001$). Peak alpha2 ERD values in the mid-prefrontal cortex (BA 9) nearly reached statistical significance (CIP: −5.8 ± 6.3%; nPE: −0.19 ± 3.3%; $t = -3.12$, $P = 0.004$). Because the epilepsy groups markedly differed in task performance, with patients with CIP providing fewer correct answers than those with nPE (Fig. 3), we tested whether these differences in power changes remained constant during equivalent task performance. For that purpose, we reanalyzed the data after excluding patients with CIP with correct-response accuracy < 90% ($N = 4$). This exploratory analysis showed that patients with CIP and nPE performed equally well ($t(20) = -1.68$, $P = 0.127$). As a result, the statistical Brain Voyager maps showed that alpha ERS source in the precuneus (values at t_{maxima}: CIP 6.2 ± 7.4%; nPE −2.9 ± 5.7%; $t = 2.97$, $P = 0.012$) and posterior parietal cortex (values at t_{maxima}: CIP 4.0 ± 4.9%; nPE −4.0 ± 6.2%; $t = 3.28$, $P = 0.007$) disappeared and no longer differed between patients with CIP and nPE; only alpha source-power changes in the right DLPFC and the left temporal cortex remained significant ($P < 0.001$) (Table 2). No significant power changes in the alpha or any other frequency band emerged after the patients with lower task performance were excluded.

A comparison between patients with schizophrenia and healthy controls revealed significantly different power changes, especially in alpha-oscillatory activity, but also, to a lesser extent, in the theta

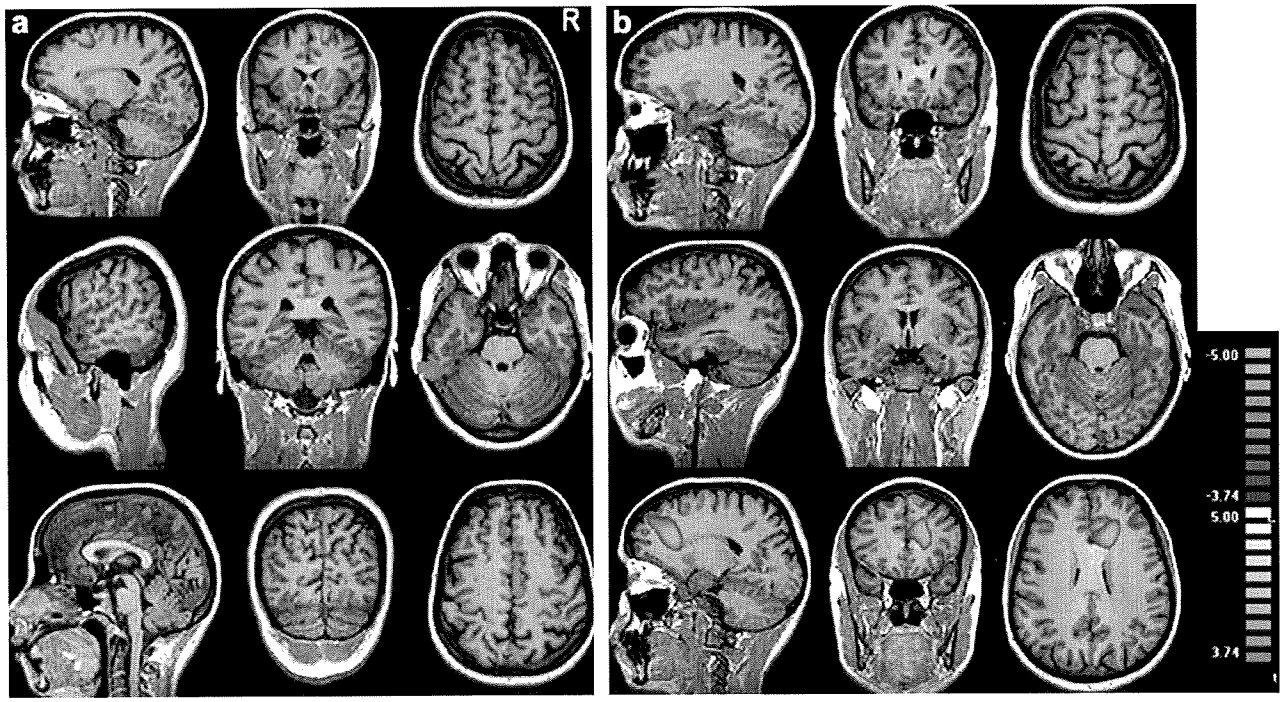

Fig. 5. Statistical maps showing cortical regions with significant between-group differences in power changes in the alpha band during working memory retention in (a) patients with CIP versus patients with nPE and (b) patients with schizophrenia versus healthy controls projected onto a Talairach-transformed T1-weighted anatomical MRI (threshold: $t = 3.74$, $P < 0.001$ uncorrected). The color bars represent the percentages of decrease (blue/green, ERD) and increase (red/yellow, ERS) in oscillatory activity power changes. R, right. (a, bottom panel) The alpha ERS disappeared after exploratory analysis excluding low-performing patients with chronic interictal psychosis. (b, bottom panel) The alpha ERD in the mid-prefrontal cortex was observed in the alpha2 subband only.