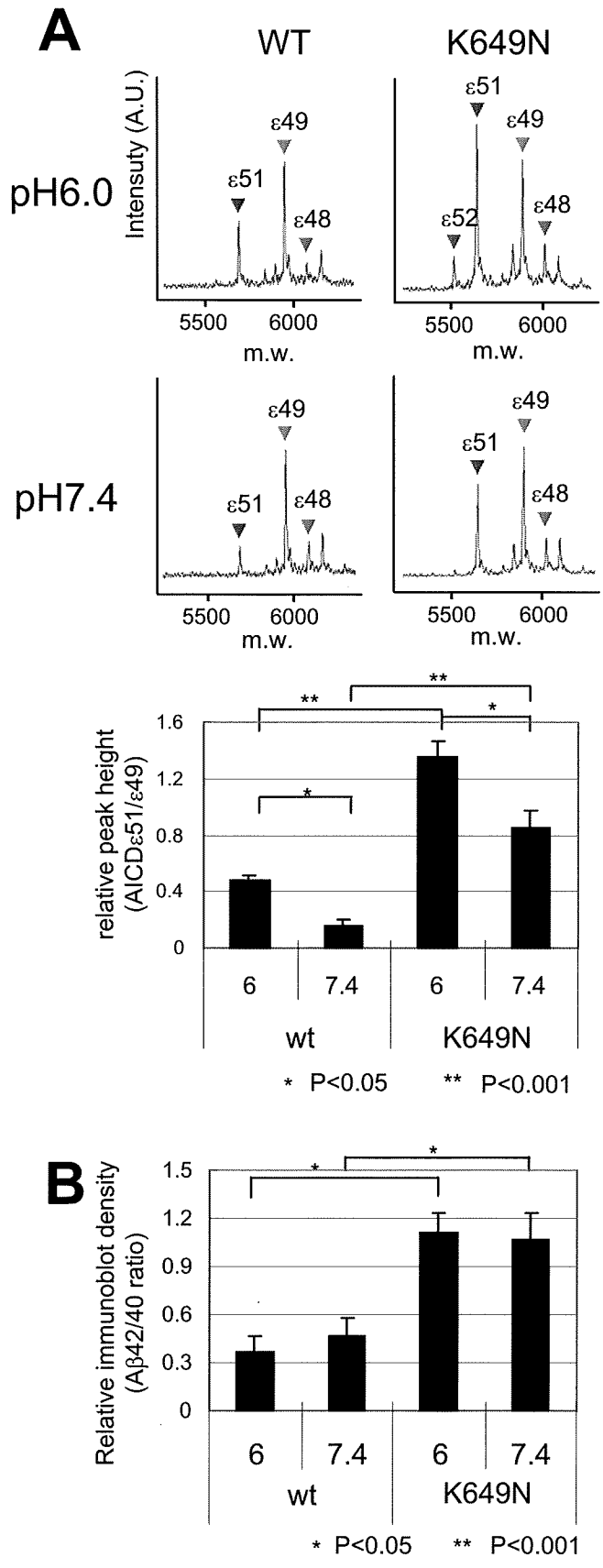
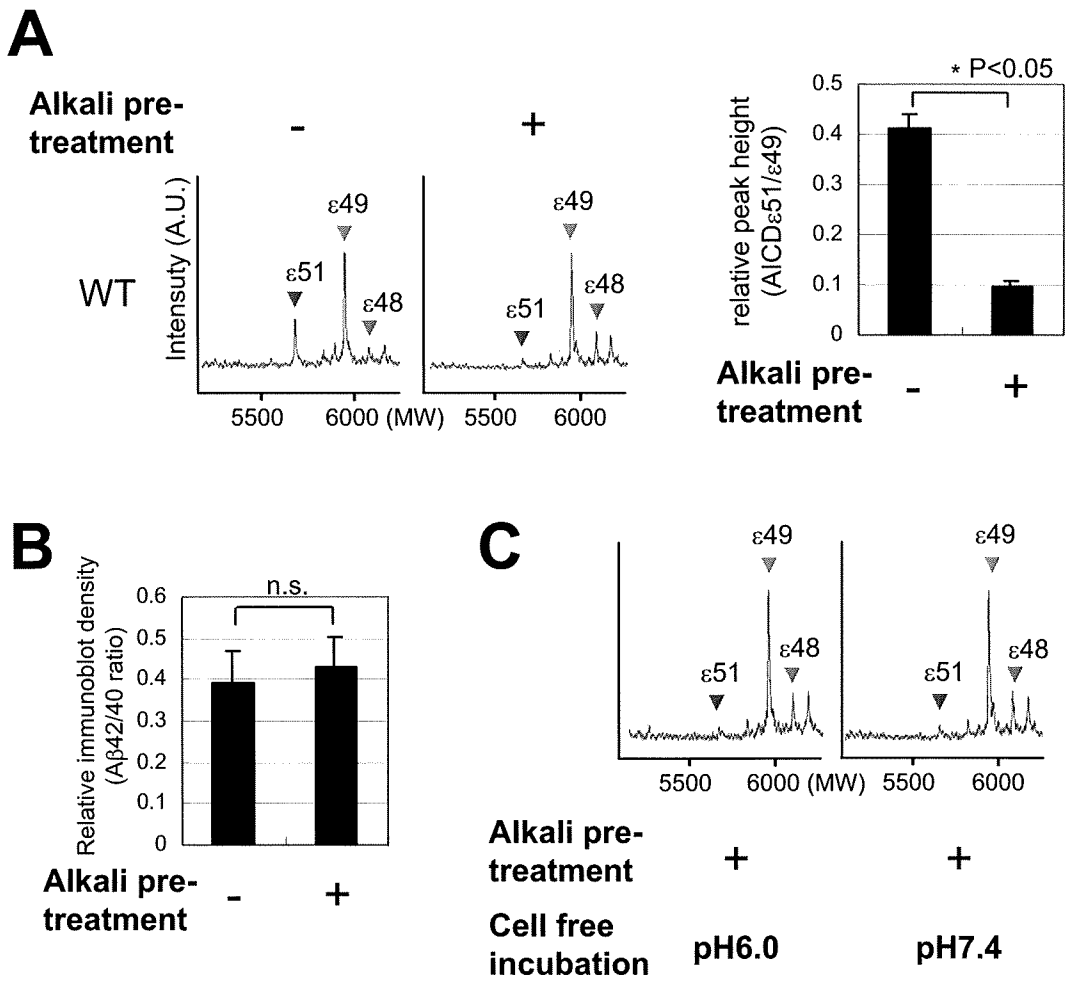
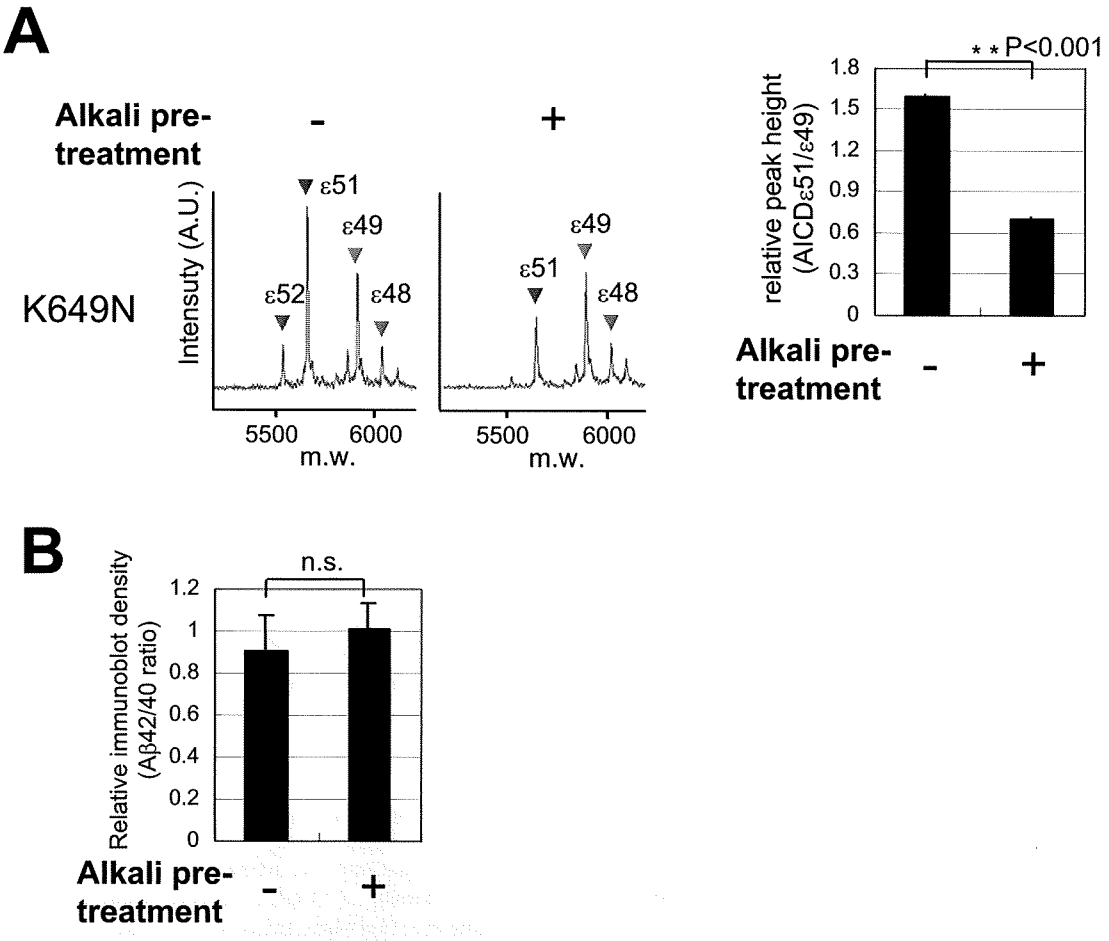


Mori et al., Figure 2



Mori et al., Figure 3



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The International Consortium on Lithium Genetics (ConLiGen): An Initiative by the NIMH and IGSLI to Study the Genetic Basis of Response to Lithium Treatment

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Manic-depressive illness · Schizoaffective disorder · Mood stabilizer · Antidepressants · Suicidal behavior · Genome-wide association study · Neurogenesis · Neuroplasticity

Abstract
For more than half a decade, lithium has been successfully used to treat bipolar disorder. Worldwide, it is considered the first-line mood stabilizer. Apart from its proven antimanic and prophylactic effects, considerable evidence also suggests an antisuicidal effect in affective disorders. Lithium is also effectively used to augment antidepressant drugs in the treatment of refractory major depressive episodes and prevent relapses in recurrent unipolar depression. In contrast to many psychiatric drugs, lithium has outlasted various pharmacotherapeutic 'fashions', and remains an indispensable element in contemporary psychopharmacology. Nevertheless, data from pharmacogenetic studies of lithium are comparatively sparse, and these studies are generally characterized by small sample sizes and varying definitions of response. Here, we present an international effort to elucidate the genetic underpinnings of lithium response in bipolar disorder. Following an initiative by the International Group for the Study of Lithium-Treated Patients (www.IGSLI.org) and the Unit on the Genetic Basis of Mood and Anxiety Disorders at the National Institute of Mental Health, lithium researchers from around the world have formed the Consortium on Lithium Genetics (www.ConLiGen.org) to establish the largest sample to date for genome-wide studies of lithium response in bipolar disorder, currently comprising more than 1,200 patients characterized for response to lithium treatment. A stringent phenotype definition of response is one of the hallmarks of this collaboration. ConLiGen invites all lithium researchers to join its efforts.

Background

The articles in this special issue of *Neuropsychobiology* comprehensively review the use of lithium as a mood stabilizer in bipolar and unipolar affective disorders. They show that 60 years after Cade's discovery, lithium is still a first-line choice for prophylaxis in bipolar disorder. They furthermore discuss the evidence regarding lithium's antisuicidal effects, its use as an augmentation strategy in the treatment of unipolar depression, and provide novel insights into its neurobiological mechanisms of action. Finally, current pharmacogenetic knowledge about lithium treatment is reviewed. Taken together, however, these articles also highlight that, despite decades of lithium use in psychiatry and despite the current emphasis on the study of psychiatric genetics in modern biological psychiatry, pharmacogenetic data regarding lithium treatment have a tendency to be circumstantial and inconclusive.

Pharmacogenetics is a rapidly growing field that holds considerable promise for the development of medications that are more personalized and effective than those currently available. In all areas of medicine, pharmacogenetic studies of outcomes such as treatment response or characteristic side effects are on the rise; based on these findings, more and more pharmacogenetic tests are being offered and approved by the US Food and Drug Administration [1]. Pretreatment genetic testing has now even been added to the prescribing information for the anticoagulant warfarin [2]. Similarly, the Food and Drug Administration updated labeling for carbamazepine, recommending that patients of Asian ancestry be screened for the presence of the HLA allele B*1502 that has been implicated in carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese people [3].

Sufficiently large, well-characterized samples as well as effective and efficient collaboration between academia and the pharmaceutical industry are among the critical prerequisites for success in the field of pharmacogenetics [4, 5]. Pharmacogenetic research in psychiatry has long been characterized by single lab efforts and small sample sizes. Only recently has our field witnessed large collaborative studies such as the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (http://www.edc.pitt.edu/stard/) [6] in the United States, or the Genome-Based Therapeutic Drugs for Depression (GENDEP) project (http://gendep.iop.kcl.ac.uk/results.php) [7] in Europe, both of which study the pharmacogenetics of major depression. Indeed, the STAR*D and GENDEP projects have already generated several intriguing findings concerning the genetics of treatment response and side effects [8–14]. It is hoped that genome-wide association studies (GWAS) conducted in these and other samples will significantly increase our ability to guide the pharmacological treatment of psychiatric patients through the identification of genetic markers.

Notably, despite lithium's proven efficacy [15], to date there has been only one GWAS examining this 'pharmacological workhorse' of psychiatry [16]. In two cohorts encompassing more than 800 lithium-treated patients,

multiple regions of interest were identified but none met the threshold for genome-wide significance. While intriguing, no adequately powered cohort yet exists to replicate and extend these findings. Here, we present a worldwide effort to address this situation: the international Consortium on Lithium Genetics (ConLiGen), spearheaded by researchers from the International Group for the Study of Lithium-Treated Patients (IGSLI) and the National Institute of Mental Health (NIMH).

The International Group for the Study of Lithium-Treated Patients

The IGSLI is an international group of scientists dedicated to lithium-related research, and its use in mental illness and mood disorders in particular. Founded in 1988 by Mogens Schou (Risskov/Aarhus, Denmark), Bruno Müller-Oerlinghausen (Berlin, Germany), and Paul Grof (Ottawa, Canada), the IGSLI has significantly contributed to lithium research over the past 20 years (www.igsli.org). Other scientists and centers have since joined the group, which currently comprises 35 members from Austria, Canada, the Czech Republic, Denmark, Germany, Poland, Switzerland, and the United States. The main goal of this group has been to conduct systematic work on those key questions regarding lithium treatment that can only be resolved by joint international effort. Unified designs have been created and scientific data from the IGSLI member centers have been linked for the purpose of shared analysis. This approach allows investigators to work with large numbers of prospectively followed patients – something that could only be accomplished via a multicenter approach. Overall, IGSLI research is based on shared, standardized, computer-based documentation of patients' diagnoses, family histories, course of illness before and during treatment, and on comparable modalities of treatment. The group meets regularly at research conferences to plan and discuss joint projects and to prepare publications.

At the 21st IGSLI meeting, which took place in late September 2007 in Dresden, Germany, the group discussed the results from the first, newly released GWAS of bipolar disorder, performed by researchers from the NIMH and Germany [17]. The strongest findings identified and replicated in this study were those encoding diacylglycerol kinase eta, a key protein in the lithium-sensitive phosphatidylinositol pathway and several genes in the *Wnt*-signaling cascade. Given the absence of a hypothesis-driven selection of single nucleotide polymorphisms in GWAS – a method more typical of candidate gene association studies – the observation that these findings implicated pathways relevant to lithium's mechanism of action was particularly intriguing. Spurred on by these findings, the IGSLI researchers concluded that studying these genes in samples that included data on patient response to lithium treatment could improve our understanding of how these genes determine response to lithium treatment and impact susceptibility to bipolar disorder. The IGSLI collaborators thus agreed to explore a framework that would allow researchers to engage in genetic studies of lithium response that were sufficiently powered. It was stated that such an endeavor should allow for participation by all bona fide lithium researchers within and beyond the IGSLI, while maintaining the highest possible level of stringency regarding phenotype definition.

May 6, 2008: The Consortium on Lithium Genetics Is Born

Following an invitation by IGSLI members Thomas G. Schulze and Francis J. McMahon, both from the NIMH's Unit on the Genetic Basis of Mood and Anxiety Disorders, prominent scientists in the field of lithium and bipolar genetic research met at the NIMH to discuss the possibility of creating an international consortium dedicated to the study of lithium pharmacogenetics. In attendance were (in alphabetical order): Martin Alda (Halifax, N.S., Canada), Michael Bauer (Dresden, Germany), Maria Del Zompo (via phone from Cagliari, Italy), Gonzalo Laje (Bethesda, Md., USA), Francis J. McMahon (Bethesda, Md., USA), Mirko Manchia (Cagliari, Italy), Roy H. Perlis (Boston, Mass., USA), Janusz K. Rybakowski (Poznan, Poland), Thomas G. Schulze (Bethesda, Md., USA), Johannes Schumacher (Bethesda, Md., USA), and Jordan W. Smoller (Boston, Mass., USA).

Reviewing evidence from the literature, and based on their own observations, the group emphasized the evident familiality in lithium treatment response, raising the possibility that genetic variation may contribute to interindividual differences in treatment response. If such differences could be identified, they might facilitate the development of novel treatments for bipolar disorder, or allow for better matching between patients and treatments. Over the last decade, the quest for a 'personalized medicine' approach in psychiatry has propelled a host of pharmacogenetic studies. Because of the lengthy trial-and-error process that currently characterizes the search

for the most optimal treatment, pharmacogenetic studies in psychiatry have traditionally focused on treatment response or adverse effects associated with antidepressants or antipsychotic medications [18–22]. While initially limited by small sample sizes, pharmacogenetic studies in psychiatry have increasingly come to rely on large-scale collaborative efforts, such as STAR*D, GENDEP, or the Clinical Antipsychotic Trials of Intervention Effectiveness project. While some pharmacogenetic studies performed with these collaborative samples have produced intriguing results, difficulties in defining stringent target phenotypes across the various subsamples remain an important challenge [23, 24].

The researchers gathered at the NIMH on May 6, 2008 noted that, despite considerable and well-documented worldwide experience with lithium as an effective antimanic agent, mood stabilizer, and putative antisuicidal agent, there is a surprising dearth of large-scale pharmacogenetic studies of lithium treatment. We thus decided to create an international initiative whose goal would be to facilitate high-quality, well-powered analyses of lithium treatment response data that would ultimately allow for robust conclusions. The Consortium on Lithium Genetics, hereafter referred to as ConLiGen, was born.

ConLiGen's Scientific Goals

ConLiGen aims to identify genetic determinants of response to lithium treatment in bipolar disorder, as well as genetic determinants of adverse events emerging during lithium treatment. In the long run, ConLiGen may also study response to lithium treatment in general (e.g. lithium augmentation in the treatment of major depression).

Membership in ConLiGen

Any bona fide researcher or research group with access to samples of lithium-treated patients for whom DNA is available can join ConLiGen. Any new admission request is voted upon by ConLiGen members.

Communication between the ConLiGen Members

To ensure a constant exchange of ideas between members and allow for a straightforward realization of ConLiGen's goals, a monthly conference call is conducted. Furthermore, members meet once or twice a year at international meetings of various biological psychiatric organizations.

ConLiGen Advisory Board

An Advisory Board comprising international experts in the field of mood disorders research, and lithium research in particular, was established to offer ConLiGen an outside perspective as well as guidance on broad scientific directions, to serve as a liaison to nonacademic communities such as funding institutions, or industry, and finally, to act as one of ConLiGen's publicly visible faces. Currently, the following researchers are members of the Advisory Board (in alphabetical order): Robert H. Belmaker (Division of Psychiatry, Ben Gurion University of the Negev, Beersheva, Israel), Gian Luigi Gessa (Department of Neuroscience 'B.B. Brodie', University of Cagliari, Cagliari, Italy), Paul Greengard (Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, N.Y., USA), Kay R. Jamison (Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Md., USA), Richard S. Jope (Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, Ala., USA), Husseini K. Manji (CNS & Pain, Johnson and Johnson Pharmaceutical Research and Development, Titusville, N.J., USA), and Leon E. Rosenberg (Department of Molecular Biology and the Woodrow Wilson School of Public and International Affairs, Princeton University, Princeton, N.J., USA).

Phenotype Definition of Lithium Response: A Major Prerequisite for Pharmacogenetic Studies of Lithium

ConLiGen's first and most crucial goal is to define the phenotype of lithium response. Treatment response is a complex construct that requires researchers to make judgments about adequacy of treatment and tolerability as well as assess changes in episode frequency or symptom severity. In many cases this information must be assessed retrospectively, with the inherent limitations associated with recall bias, missing information, or the fact that the treatment has not followed a strict research protocol. One scale that incorporates such data is an 11-point scale developed by Martin Alda and colleagues [25]

(fig. 1); other approaches include longitudinal outcome measures that consider time to recurrence or symptom burden during treatment [17, 26].

The 11-point scale measures the extent of improvement during long-term treatment. The scale's A score is a composite measure of change in frequency, duration, and severity of illness episodes in the course of lithium treatment. It is weighted by factors that influence the degree to which the observed clinical change is considered to be due to lithium (B1–B5 scores in the scale). The scale has been developed in the context of a study assessing response to treatment in subjects not followed according to a research protocol, namely relatives of probands in our genetic studies [25]. Subsequently, it has been widely used in several other studies at IGSLI centers [27–29] and at other centers involved in lithium research [pers. commun. from John Kelsoe, San Diego, Calif., USA and Maria del Zompo, Cagliari, Italy], which imparts face validity. Within ConLiGen, phenotypic assessment will be based on any available information including life charts when available and quantified using the scale; interrater reliability meetings will be organized, facilitated by ConLiGen member Martin Alda, and case vignettes will also be reviewed to establish between-center reliability.

Variables describing treatment tolerability or side effects may be studied in subsequent projects. Because the issue of 'best response phenotype' is far from trivial, ConLiGen will strive to continuously weigh evidence from future clinical and biological studies of lithium in an effort to refine the definition of phenotype response. Evaluating response to long-term treatment in an illness with a highly variable natural course presents a challenge. Many patients with bipolar disorder experience spontaneous remissions of variable timing and duration. Moreover, in a pharmacogenetic study we need to evaluate the quality of response not for groups of subjects as in clinical trials but individually for each patient. While prospective studies will be able to implement more precise measures, our approach is a practical way to assess the quality of response in a variety of patients treated in diverse settings.

ConLiGen's Current Project and Long-Term Mission

ConLiGen is poised to assess all aspects of the pharmacogenetics of lithium treatment in psychiatric disorders, including the study of genetic susceptibility to potential treatment-emergent adverse events (e.g. weight gain, hypothyroidism, tremor). As its first project, ConLiGen intends to conduct a GWAS of stringently defined response to lithium treatment in bipolar disorder. ConLiGen members and the various research centers which they are affiliated with are joining their samples for a centralized genotyping effort to be performed at the Unit on the Genetic Basis of Mood and Anxiety Disorders of the NIMH. For the primary projects, a previously validated scale will be used to define response to lithium treatment, as described above. Individuals scoring between 7 and 10 will be considered lithium 'responders', while individuals with scores between 0 and 6 will be considered 'nonresponders'. Presently, the total sample comprises more than 1,200 bipolar patients for whom response to lithium treatment has been or is currently being assessed by means of the scale. From preliminary analyses conducted in select IGSLI samples (data not shown), we can assume that about 35–40% of patients will qualify as responders. Previous studies [8, 9] suggest larger genetic effect sizes (e.g. allelic odds ratios between 1.5 and 2) for a narrowly defined pharmacogenetic phenotype than for a categorically defined clinical diagnosis. Thus, assuming a minor allele frequency of 0.3 and genotype relative risks of 1.4 for individuals heterozygous, and of 1.96 for individuals homozygous for the risk allele, the combined ConLiGen sample will have a power of 83% to detect an effect at a significance level of 1×10^{-8} [30].

Although the combined ConLiGen sample will be the largest sample to date to investigate lithium response on a genome-wide scale, we are aware that any finding, regardless of whether it reaches levels of genome-wide significance, will ultimately have to be confirmed in independent samples. Thus, ConLiGen's mission will not be finished after the completion of its GWAS. On the contrary, ConLiGen will continue to invite researchers to join its efforts in order to increase the available sample size of patients adequately characterized for lithium response. In collaboration with both IGSLI centers and large, long-standing multicenter projects such as the NIMH Bipolar Disorder Genetics Initiative, ConLiGen will be actively engaged in supporting and organizing urgently needed prospective studies of lithium response in bipolar disorder and other conditions.

Since Cade discovered lithium's beneficial effects in the treatment of bipolar disorder 60 years ago, this agent has become almost synonymous with the treatment of bipolar disorder worldwide [15]. Yet, little is known about the genetic underpinnings of lithium response or the development of side effects associated with its use. In a scientific environment characterized by calls for personal-

ized medicine and the growth of large-scale pharmacogenetic studies in many fields of medicine, ConLiGen's goal is to put lithium at the forefront of pharmacogenetic studies in psychiatry.

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ORIGINAL PAPER

Association Study Between the Pericentrin (*PCNT*) Gene and Schizophrenia

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Abstract Disrupted-in-schizophrenia 1 (*DISC1*), a known genetic risk factor for schizophrenia (SZ) and major depressive disorder (MDD), interacts with several proteins and some of them are reported to be genetically associated with SZ. Pericentrin (*PCNT*) also interacts with *DISC1* and recently single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show significant associations with SZ and MDD. In this study, case-controlled association analysis was performed to determine if the *PCNT* gene is implicated in SZ. Nine SNPs were analyzed in 1,477 individuals (726 patients with SZ and 751 healthy controls). No significant difference was observed between the controls and the patients in allelic frequencies or genotypic distributions of eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects, either. This gene may not play a major role independently in the etiology of SZ in the Japanese population.

Keywords Schizophrenia · PCNT · Kendrin · Case–control association study · DISC1

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Introduction

Schizophrenia (SZ) is a complex psychiatric disorder that afflicts approximately 1% of the population throughout the world and has high heritability (Craddock et al. 2005). The pericentrin gene (the official symbol; *PCNT* and also called kendrin) is located at 21q22.3, which is one of chromosomal lesions prevalent in SZ by cytogenetic analysis (Demirhan and Tastemir 2003). *PCNT* is a coiled-coil protein localized specifically to the centrosome throughout the cell cycle (Flory et al. 2000) and an integral component of the pericentriolar material (Li et al. 2001). This protein provides sites for microtubule nucleation in the centrosome by anchoring gamma-tubulin complex (Takahashi et al. 2002), then it plays an important role in microtubule organization, spindle organization, and chromosome segregation (Doxsey et al. 1994; Purohit et al. 1999). Disrupted-in-schizophrenia 1

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(*DISC1*), a known genetic risk factor for SZ and major depressive disorder (MDD) (Cannon et al. 2005; Chen et al. 2007; Hashimoto et al. 2006; Hennah et al. 2003; Millar et al. 2000; Thomson et al. 2005), localizes to the centrosome by binding to *PCNT* (Miyoshi et al. 2004). Shimizu et al. showed that overexpression of the *DISC1*-binding regions of *PCNT* or the *DISC1* deletion mutant lacking the *PCNT*-binding region impaired the microtubule organization and they suggested that the *DISC1–PCNT* interaction played a key role in the microtubule network formation (Shimizu et al. 2008). Recently, single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show allelic associations with SZ and MDD (Anitha et al. 2009; Numata et al. 2009). In addition, Mitkus et al. reported a trend for an increase mRNA levels of the *PCNT* gene in the dorsolateral prefrontal cortex of patients with SZ, compared with the control groups (Mitkus et al. 2006). In this study, case-controlled association analysis was performed in the Japanese population to determine if the *PCNT* gene is implicated in SZ.

Materials and Methods

Subjects

We used genomic DNA samples from 726 SZ patients: 406 male (mean age 48.6 ± 13.8 years), 320 female (mean age 49.2 ± 14.5 years) from the Tokushima University Hospital, affiliated psychiatric hospitals of the University of Tokushima, the Ehime University Hospital and the Osaka University Hospital in Japan. The diagnosis of SZ was made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of extensive clinical interviews and review of medical records. Seven hundred fifty-one controls, 422 male (mean age 45.5 ± 11.1 years) and 329 female (mean age 45.2 ± 10.5), were selected from volunteers who were recruited from hospital staff and students and company employees documented to be free from either psychiatric problems or past mental histories. All subjects were unrelated Japanese origin and signed written informed consent to participate in the genetic association studies approved by the institutional ethics committees.

Genotyping

We initially selected eight tagging SNPs by SNPBrowser 3.5 (De La Vega et al. 2006) (Applied Biosystems, Foster, CA, USA, Pair-wise $r^2 > 85\%$, MAF > 20%, Japanese population) (rs11702684, rs2249057, rs11701058, rs2839226, rs2839231, rs3788265, rs2073376, rs1010111) (Supplementary Table 1). After that, we selected rs2073380 additionally because eight tagging SNPs did not seem to cover the third block of the *PCNT* gene from HapMap data. Genotyping was performed using commercially available TaqMan probes for the *PCNT* gene with ABI Prism 7900 HT Sequence Detection System and ABI 7500 Real Time PCR System (Applied Biosystems). Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al. 2005). Blocks were defined according to the criteria of Gabriel et al. (2002).

Statistical Analysis

Allelic and genotypic frequencies of patients and control subjects were compared using χ^2 test. The SNPAlyze 3.2Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, linkage disequilibrium (LD), permutation *P*-values (10,000 replications) and deviation from Hardy–Weinberg Equilibrium (HWE) distribution of alleles. Power calculations for our sample size performed using the G*Power program (Erdfelder et al. 1996). The criterion for significance was set at $P < 0.05$ for all tests.

Results

Genotypic and allelic frequencies of the *PCNT* gene are shown in Table 1. Genotypic distributions of these nine SNPs did not deviate significantly from HWE in either group ($P > 0.05$). No significant difference was observed in genotypic frequency between the controls and patients in eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In power calculations using the G*Power program, our sample size had >0.98 power for detecting a significant association (alpha < 0.05) when an effect size index of 0.2 was used.

Several papers reported that there were gender-specific genetic components involved in the pathology of SZ in the *DISC1* gene (Hennah et al. 2003; Chen et al. 2007) and the *DISC1*-related genes (Hennah et al. 2007; Pickard et al. 2007; Qu et al. 2008). In our study, when the data were subdivided on the basis of gender, allelic distribution of rs11702684 was different between the two groups in only male samples ($P = 0.033$). However, the difference did not survive statistical significance after permutation correction for multiple comparisons.

There were three LD blocks in the *PCNT* gene with rs2249057, rs11701058, rs2839226, and rs2839231 residing in block 1 and rs3788265 and rs2073376 residing in block 2, and rs2073380 and rs1010111 residing in block 3 (Gabriel et al. 2002, Fig. 1). These constructed marker haplotypes of blocks 1–3 were not associated with SZ (permutation $P = 0.184$, 0.137, and 0.601, respectively).

Table 1 Genotypes and allele frequencies of nine single SNPs in the PCNT gene in patients with SZ and controls

SNP	Diagnosis	Allele		*P*-value	Genotype			*P*-value	Frequency
rs11702684		C	T		C/C	C/T	T/T		
	SC	913	515	0.042	296	321	97	0.085	0.361
	CT	892	588		265	362	113		0.397
		C	A		C/C	C/A	A/A		
rs2249057	SC	862	590	0.504	255	352	119	0.691	0.406
	CT	870	626		247	376	125		0.418
		C	T		C/C	C/T	T/T		
rs11701058	SC	669	783	0.181	153	363	210	0.297	0.461
	CT	728	772		168	392	190		0.485
		C	T		C/C	C/T	T/T		
rs2839226	SC	378	1072	0.111	47	284	394	0.19	0.261
	CT	353	1147		34	285	431		0.235
		A	G		A/A	A/G	G/G		
rs2839231	SC	408	1042	0.562	63	282	380	0.52	0.281
	CT	405	1085		53	299	393		0.272
		G	T		G/G	G/T	T/T		
rs3788265	SC	821	627	0.998	234	353	137	0.506	0.433
	CT	846	646		230	386	130		0.433
		A	G		A/A	A/G	G/G		
rs2073376	SC	445	1001	0.403	75	295	353	0.51	0.308
	CT	478	1006		77	324	341		0.322
		C	A		C/C	C/A	A/A		
rs2073380	SC	642	796	0.839	144	354	221	0.552	0.446
	CT	669	817		141	387	215		0.45
		A	G		A/A	A/G	G/G		
rs1010111	SC	1079	363	0.298	402	275	44	0.343	0.252
	CT	1141	351		428	285	33		0.235

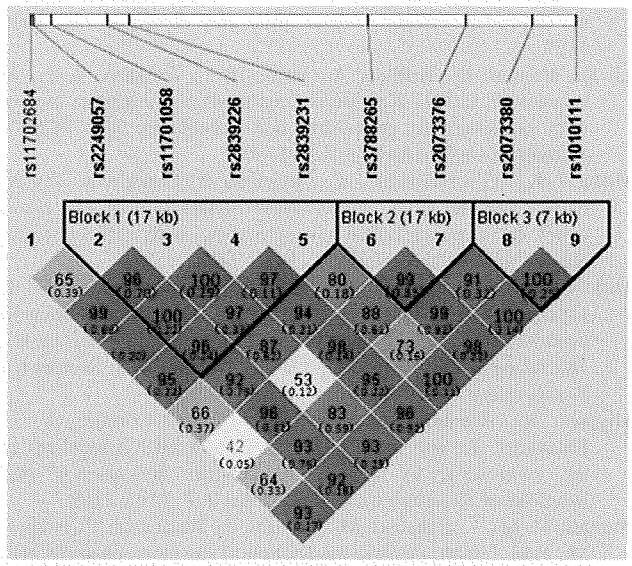

Fig. 1 LD and haplotype structure of the PCNT gene. Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al. 2005). Blocks were defined according to the criteria of Gabriel et al. (2002). Each box represents the D' (r^2) values corresponding to each pair-wise SNP

Discussion

In this study, we examined the association of nine SNPs in the *PCNT* gene and SZ. No significant difference was observed between the controls and the patients in either allelic frequencies or genotypic distributions of nine SNPs after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects. This result was concordance with another study in a Caucasian population (Tomppo et al. 2009).

During the preparation of this article, Anitha et al. reported that rs2249057 of the *PCNT* gene and haplotypes involving this SNP were significantly associated with SZ after correction for multiple comparisons in the Japanese population (Anitha et al. 2009). Although SNPs examined in our study contained rs2249057, we could not find any significant associations in our subjects. The statistical power of our study was sufficient to detect an association between the variants and SZ (SZ $n = 726$; control $n = 751$). Surprisingly, the control minor allele frequency of rs2249057 in Anitha's study (0.48) was higher than

those of our study, HapMap data, and ABI data (0.42, 0.40, and 0.41, respectively). This differing allele frequency between these two studies may be caused by samples' recruited areas. Anitha et al. used subjects from further east compared to ours. However, it is reported that there is no significant population stratification in Japanese (Arinami et al. 2005; Yamaguchi-Kabata et al. 2008).

There are several limitations in our study. First, we applied MAF > 20% when we selected the tagging SNPs and it is difficult to evaluate the association of rare variants in our study. Second, we cannot rule out a possibility that *DISC1–PCNT* interaction may be involved in the etiology of SZ. Third, our findings only represented the Japanese population and studies in other populations would still be warranted due to differing allele frequencies between populations.

Conclusions

In conclusion, we did not find any significant association between the *PCNT* gene and the SZ in the Japanese population. This gene may not play a major role independently in the etiology of SZ.

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Discriminant analysis in schizophrenia and healthy subjects using prefrontal activation during frontal lobe tasks: A near-infrared spectroscopy

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ABSTRACT

While psychiatric disorders such as schizophrenia are largely diagnosed on symptomatology, several studies have attempted to determine which biomarkers can discriminate schizophrenia patients from non-patients with schizophrenia. The objective of this study is to assess whether near-infrared spectroscopy (NIRS) measurement can distinguish schizophrenia patients from healthy subjects. Sixty patients with schizophrenia and sixty age- and gender-matched healthy controls were divided into two sequential groups. The concentration change in oxygenated hemoglobin (Δ[oxy-Hb]) was measured in the bilateral prefrontal areas (Fp1-F7 and Fp2-F8) during the Verbal Fluency Test (VFT) letter version and category version, Tower of Hanoi (TOH), Sternberg's (SBT) and Stroop Tasks.
In the first group, schizophrenia patients showed poorer task performance on all tasks and less prefrontal cortex activation during all but the Stroop Task compared to healthy subjects. In the second group, schizophrenia patients showed poorer task performance and less prefrontal cortex activation during VFTs and TOH tasks than healthy subjects. We then performed discriminant analysis by a stepwise method using Δ[oxy-Hb] and task performance measures as independent variables. The discriminant analysis in the first group included task performance of TOH, VFT letter and VFT category and Δ[oxy-Hb] of VFT letter. As a result, 88.3% of the participants were correctly classified as being schizophrenic or healthy subjects in the first analysis. The discriminant function derived from the first group correctly assigned 75% of the subjects in the second group. Our findings suggest that NIRS measurement could be applied to differentiate patients with schizophrenia from healthy subjects.

1. Introduction

Major psychiatric disorders such as schizophrenia are largely diagnosed on symptomatology (World Health Organization, 1992; American Psychiatric Association, 1994). While the validity of diagnostic criteria continues to be debated, major advances have been made in understanding the biology of these disorders. However, identified biological markers of psychiatric diseases, including schizophrenia, are not currently used in their diagnosis.

Candidate biological markers of schizophrenia include pathogenetic factors, physical findings, neurophysiological and neuropsychological functioning, and structural and functional brain imaging. In particular, neuroimaging techniques hold significant advantages and have provided evidence for localized anatomical and functional abnormalities, complemented by the use of cognitive neuroscience (Abou-Saleh, 2006). Abnormalities in the prefrontal cortex as well as other brain regions (Arnold and Trojanowski, 1996; Bogerts et al.,

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1990; Carter et al., 1998; Harrison, 2005; Heckers et al., 1998; Laurens et al., 2005; Torrey, 2007) and connections between these regions (Fletcher, 1998; Volkow et al., 1988; Weinberger et al., 1992) have been identified as substrates of the clinical features of schizophrenia. For example, positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies identified prefrontal cortex dysfunction as one of the characteristic features of the disease (Callicot et al., 2000; Carter et al., 1998; Curtis et al., 1998). These studies highlight the prefrontal cortex as a promising brain region in the potential use of functional imaging as a biological marker of psychiatric disorders.

Near-infrared spectroscopy (NIRS), a brain functional measuring technique, can measure changes in the concentration of oxygenated Hemoglobin ([oxy-Hb]), deoxygenated Hemoglobin ([deoxy-Hb]) and total Hemoglobin ([total-Hb]), which are presumed to reflect regional cerebral blood flow (Jobsis, 1977). These hemodynamic parameters are assumed to be a stable marker of cerebral oxygenation changes induced by cognitive tasks (Nakahachi et al., 2008; Suto et al., 2004; Takizawa et al., 2008;). NIRS is advantageous for clinical application over other neuroimaging techniques such as PET, SPECT and functional magnetic resonance imaging (fMRI), due to its non-invasive nature, high time resolution, portable and simple mounting, low cost, robustness against motion artifacts, short time of measurement and little training required for operation and data analysis. Therefore, the prefrontal dysfunction in schizophrenia has been frequently investigated with NIRS in a clinical setting. Studies in the frontal cortex have demonstrated a significant smaller increase in the prefrontal activation during the Verbal Fluency test (Ikezawa et al., 2009; Kubota et al., 2005; Suto et al., 2004; Takizawa et al., 2008; Watanabe and Kato, 2004), the Random Number Generation task (Hoshi et al., 2006) and the Tower of Hanoi task (Ikezawa et al., 2009) in patients with schizophrenia compared to healthy control subjects. However, patients with schizophrenia showed no differences during divergent thinking task (Folley and Park, 2005), and possibly a larger increase during the unilateral finger tapping task (Suto et al., 2004) or letter number span test (Watanabe and Kato, 2004). These studies suggest that NIRS measurement of frontal lobe activity may represent a biological marker of schizophrenia on which frontal lobe tasks are employed.

Despite the potential benefit of NIRS measurement of frontal lobe activity as a biological marker of schizophrenia, to our knowledge, discriminant analysis with NIRS has not previously been applied to distinguish schizophrenia patients from healthy subjects. The present study aimed to evaluate whether the NIRS measurement in the frontal cortex could reliably distinguish patients with schizophrenia from control subjects and to identify the task which would provide the highest correct classification rate.

2. Methods

2.1. Subjects

Subjects were assigned to two independent groups according to the order of study inclusion. The first group consisted of a total of 60 subjects, including 30 patients with schizophrenia and 30 age- and gender-matched healthy control subjects. The period of study for this group was from November 2006 to May 2007.

The second group for the prospective validation also consisted of 60 subjects: 30 with schizophrenia and 30 age- and gender-matched healthy control subjects. The period of study for this group was from June 2007 to April 2008.

The patients were inpatients and outpatients of the Department of Psychiatry, Osaka University Hospital. Each patient underwent a Structured Clinical Interview for DSM-IV (SCID) (First et al., 2002), and two or more experienced psychiatrists reached a consensus diagnosis of schizophrenia according to the DSM-IV (American Psychiatric Association, 1994) and ICD-10 for research (World Health Organization, 1992) on the basis of SCID and all other sources of clinical data. At the time of study, in the first group, 2 patients were medication naïve and 28 patients were medicated (2 patients were receiving typical antipsychotics, 11 patients were receiving atypical and 15 patients were receiving both types of antipsychotics). In addition, 11 patients were taking anxiolytics and 2 patients were taking antidepressants. In the second group, all patients (except one from which medication data was missing) were on medication (4 patients were receiving typical antipsychotics, 14 patients were receiving atypical antipsychotics and 11 patients were receiving both types of antipsychotics). In addition, 23 patients in the second group were taking anxiolytics and 3 were taking antidepressants (Table 1).

Advertisements were posted at local hospitals to recruit healthy subjects. Healthy subjects were diagnostically interviewed and assessed to verify that they had neither personal nor family history of psychiatric disease, and had taken no antipsychotics. All of the healthy subjects had at least no fourth-degree relative with a psychiatric disorder and had an estimated IQ of 70 or greater. All patients were physically healthy at the time of recruitment, and none had a history of head trauma, serious medical or surgical illness, or alcohol/substance abuse disorder. All procedures were approved by the ethical committee of the Osaka University hospital.

All participants provided written informed consent according to the Declaration of Helsinki after they were given a complete explanation of the study procedures.

2.2. Tasks and procedure

The cognitive paradigm employed in the present study consisted of the letter and category versions of the Verbal Fluency Test (VFT), Tower of Hanoi (TOH), Sternberg's Task (SBT), and the Chinese character version of the Stroop Task part III (SRT). These frontal activation tasks comprised a 30-s pre-task baseline period, a 60-s or 120-s task period, and a 60-s post-task baseline periods (Fig. 1). These procedures were similar to that of Suto et al. (2004), Ito et al. (2005) and Kameyama et al. (2006) except for the use of a 120-s task period for SBT or STR instead of the 60-s used in their studies. We used a longer interval for these two tasks to enable a more satisfactory [oxy-Hb] activation compared to the baseline in the pre-task period. This is described in detail elsewhere (Ikezawa et al., 2009).

2.2.1. Verbal Fluency Test letter and category versions

For the pre- and post-task baseline periods of the VFT letter and category versions, the subjects were instructed to repeat the voice vowels (/a/, /i/, /u/, /e/ and /o/ (Phonetic Alphabet)) constantly. During the VFT periods, they were instructed to alternately produce as many Japanese nouns as possible

Table 1
Clinical characteristics of the study groups.

Variable	Schizophrenia, $n=30$	Control, $n=30$	Group difference	*df*	*t*
	Mean(SD)	Mean(SD)	*P value*		
a. First group					
Age (year)	38.7 (11.7)	37.3 (8.7)	.601	58	−0.53
Gender Male/Female	12/18	13/17	.793*	1	
Handedness (Right/Left)	30/0	30/0	−*	1	
Education (year)	13.3(2.4)	15.5(2.1)	.001	48.23	3.51
JART premorbid IQ	100.8(10.6)	105.9(8.4)	.049	49.43	2.02
Outpatient/Inpatient	13/17	NA			
Age of onset (year)	24.3(10.2)	NA			
Medication					
Typical/Atypical/Combined (*n*)	2/11/15, 2 medication naïve	NA			
CPZeq dose (mg/day)	842.6 (704.2)	NA			
BZDeq dose (mg/day)	17.1 (11.1)a	NA			
Illness duration (year)	14.7 (13.0)	NA			
PANSS positive	18.8 (5.5)	NA			
PANSS negative	18.2 (6.4)	NA			
PANSS general psychopathology	36.8 (9.1)	NA			
b. Second group					
Age (year)	39.6 (13.1)	39.5 (13.1)	.977	58	−0.03
Gender Male/Female	16/14	18/12	.602*	1	
Handedness (Right/Left)	29/1	30/0	.313*	1	
Education (year)	14.4 (1.8)	15.5 (3.0)	.108	57	1.64
JART premorbid IQ	104.1 (10.4)	106.2 (6.8)	.356	57	0.93
Outpatient/Inpatient	21/9	NA			
Age of onset (year)	25.8 (10.8)	NA			
Medication					
Typical/ Atypical/ Combined (n)	4/14/11, 1 NA	NA			
CPZeq dose (mg/day)	881.4 (761.6)	NA			
BZDeq dose (mg/day)	18.9 (19.7)b	NA			
Illness duration (year)	13.1 (11.7)	NA			
PANSS positive	18.9 (7.5)	NA			
PANSS negative	21.1 (8.6)	NA			
PANSS general psychopathology	41.0 (11.9)	NA			

Abbreviations: JART, the Japanese version of the National Adult Reading Test; IQ, Intelligence Quotient; CPZeq, chlorpromazine equivalent; BZDeq, benzodiazepine equivalent; PANSS, the Positive and Negative Syndrome Scale; NA, not applicable.
*Chi-square test was used.
a,Eleven patients or b,23 patients received anxiolytics.

beginning with a designated syllable (/a/, /ka/, /sa/) or belonging to a certain category of words (animals, vegetables, conveyance), which is commonly used in Japanese VFT letter/category version. The three initial/categorical syllables/words were presented in an order which was counterbalanced among the subjects. These were changed every 20 s during the 60-s task period to prevent the subjects from being at a loss for words for the entire 60-s task. An auditory cue was used to change from one task period to another during the measurements. The total number of correct words generated during VFT was used as a measure of task performance.

2.2.2. Tower of Hanoi

This test consists of three or four disks of different sizes slotted onto three pegs. The subjects are given a stack of some disks, initially stacked in decreasing size on one of the three

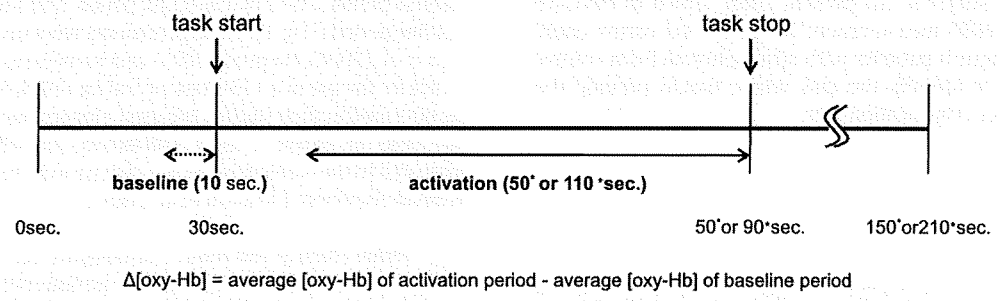

Fig. 1. Design for the sequence of five tasks with letter and category versions of the Verbal Fluency Test *, Tower of Hanoi *, Sternberg's Task ⋆ and Stroop Task ⋆. Subjects performed 150* s. or 210 ⋆ s. prefrontal tasks consisting of 30 s. pre-task period before the activation task, 60* s. or 120 ⋆ s. activation period, and 60 s. post-task period after each task. Data were sampled for the 10 s. as baseline data and for 50 * s. or 110 ⋆ s. just 10 s. after the activation task started as activation data. The difference of the average levels between activation condition and baseline was defined as the size of activation (Δ[oxy-Hb]). See text for details.

pegs. The objective is to transfer the entire tower to one of the other pegs, replicating the original stack by moving only one disk at a time and never a larger disk onto a smaller disk. The subjects are required to complete the task in as few moves as possible. The score is represented by the number of correct moves employed to transfer the entire stack (Gras-Vincendon et al., 1994). Before the recording, the subjects were sufficiently trained to perform this TOH task with three disks. The four-disk version of TOH was employed during NIRS measurement. For the pre- and post-task baseline periods, the participants were instructed to move one disk from one of the other pegs from side to side repeatedly. This was intended to correct the data during the TOH for activation due to manual movement. During the 60-s recording periods, subjects were instructed to complete the TOH task in as few moves as possible. The subjects were instructed by an auditory cue at the start and end of the task. The score of correct moves employed during TOH was used as a measure of task performance.

2.2.3. Sternberg's task

For the pre- and post-task baseline periods, of the SBT, subjects were asked to look at a point of fixation on a 15 in. monitor connected to a desktop PC placed about 1 m away from the subject. During the 120-s recording periods, the subjects performed a modified Sternberg's memory task (Sternberg, 1966) which comprised 8 trials. Each trial is as follows: a set of five different numbers is presented for 2 s; then 3 s later a series of 3 single numbers are presented for 0.8 s. The subjects were requested to answer 'yes' or 'no' if the number was included or not in the previous set. The next trial commenced 2 s later. Trial types were fixed. The subjects were instructed visually by a cue at the start and end of the task. The number of correct answer was used as a measure of task performance.

2.2.4. Stroop task part III (Chinese character version)

The Chinese character version of the Stroop Task was used (Stroop, 1935; modified according to Zysset et al., 2001; Kato, 2001). In our test, each Chinese character has the semantic value of a color. For the pre- and post-task baseline periods of the SRT, the subjects were instructed to look at a point of fixation on a PC screen. During the 120-s recording periods, Chinese characters were displayed one by one on a screen set at subject eye-level and changed every 3 s with no interval. An experimental run consisted of 40 characters (20 incongruent and 20 congruent trials) in random order. The subjects were asked to answer the color of the character printed in a color, but not the semantic meaning. Each character was randomly printed in the same color as its meaning or in a different color from its meaning. The subjects were instructed by a visual cue at the start and the end of the task. The number of correct answers was used as a measure of task performance.

2.3. NIRS measurement

NIRS measurements were conducted with a two-channel NIRS system (NIRO 200, Hamamatsu Photonics, Japan). A pair of fiber ends (light emitter and detector) was attached to the surface of the scalp to record prefrontal activation while the subjects performed the tasks in a non-invasive and minimally restrictive way. The time needed for this attachment is usually less than 20 s with little demand on the subjects. The distance between emitter and detector ends was set at 4.0 cm to estimate penetration depth of approximately 2–3 cm (Villringer and Chance, 1997), that is, the surface of the cerebral cortex (Hock et al., 1997; Okada and Delpy, 2003a,b; Toronov et al., 2001). Then the light detector was symmetrically-placed at position Fp1 and Fp2 and the emitter was placed lateral to the corresponding detector on the Fp1-F7 and Fp2-F8 line according to the international 10–20 system used in electroencephalography. These positions correspond to the inferior frontal gyrus (Homan et al., 1987, Okamoto et al., 2004), which is part of the dorsolateral prefrontal cortex.

The NIRS machine measures changes in [oxy-Hb], [deoxy-Hb] and [total-Hb] using a reflectance mode with three different wavelengths (775, 810, 850 nm) of near-infrared light based on the modified Beer-Lambert law (Cope and Delpy, 1988). This technique primarily provides data on changes in chromophore concentrations from a discretionary base line. By assuming the differential path length factor to be 24 cm, the measure of changes in the chromophore concentration corresponds to A μmol/L. The time resolution of the NIRS measurements was every 1/6 s. The subjects sat on a chair with their eyes open throughout the measurements and were asked not to move their head, legs and any part of their body unrelated to the task to reduce artifacts. Changes in [oxy-Hb] were measured during the cognitive activation tasks.

2.4. Statistical analysis

2.4.1. Group comparison

In the majority of analyses, chi-squared or *t*-test were used for group comparison ($P<.05$). Symptom levels were measured with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Most of the patients were taking psychotropic medications at the time of the study. All the participants were native Japanese speakers. Premorbid intelligence quotient (IQ) was estimated using the Japanese version of the National Adult Reading Test (JART) (Matsuoka et al., 2006).

2.4.2. Analysis of NIRS data

Since the change in [oxy-Hb] is the most sensitive indicator of rCBF changes (Hoshi et al., 2001), we evaluated the changes in [oxy-Hb] signals. In each subject, the average levels in [oxy-Hb] were calculated for both baseline and activation conditions for each task. The last 10-s of the pre-task period was defined as baseline and the 50- or 110-s of the active task period excluding the first 10-s intervals of the tasks, was defined as activation condition since stable elevation of [oxy-Hb] was usually observed several seconds after task initiation. The difference of the average levels between activation condition and baseline was defined as the size of activation (Δ[oxy-Hb]). Statistical analysis was conducted on the average of the bilateral Δ[oxy-Hb]s because Δ[oxy-Hb]s in both the right and left channels were highly correlated during the same task.

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

The data were analyzed using SPSS for Windows version 12.0 (SPSS Japan Inc., Tokyo, Japan). In the first experiment, stepwise discriminant analyses using the Wilks' lambda method were performed to distinguish schizophrenia patients from healthy controls in the first group. The criterion for inclusion/exclusion of *P* value was 0.05.

Data in 10 variables was obtained as candidate independent variables; 5 measures for the performance of each task, and 5 averaged measures of Δ[oxy-Hb] during these tasks from the left and right sides of the prefrontal areas. To verify whether the NIRS measurements could contribute to discriminate between schizophrenia patients and healthy control, we decided to investigate three combinations: task performance only (5 variables), Δ[oxy-Hb] only (5 variables) and task performance plus Δ[oxy-Hb] (10 variables). In the second experiment, we used LDA to test the validity of the discriminant functions derived from the original study prospectively in the second group.

3. Results

3.1. Group comparison

Demographic and clinical characteristics of the study groups and group comparisons are displayed in Table 1. There was no significant difference between schizophrenia patients and healthy subjects in the first or second groups apart from educational year (*t*-test; $P=.001$) and JART (*t*-test; $P=.049$) in the first group. No significant correlation between educational year and the each task performance or each NIRS measurement was found in the first and second groups. There was a significant correlation between JART and the SBT performance of healthy subjects ($r=0.392$, $P=.032$) and patients ($r=0.597$, $P=.001$) in the first group, but no significant correlation in the second group.

Comparisons of frontal task performances and NIRS measurements between schizophrenia patients and healthy subjects are displayed in Table 2. Schizophrenia patients indicated significantly poor task performances compared to healthy subjects for all five tasks in the first group and for the VFT letter, VFT category and TOH in the second group. Likewise, schizophrenia patients showed lower [oxy-Hb] increases compared to controls during the VFT letter, VFT category, TOH and SBT in the first group and during the VFT letter, VFT category and TOH in the second group.

As for the association of drugs with task performances and NIRS measurements, there was a significant correlation between chlorpromazine equivalent (CPZeq) and performance of SRT ($r=-0.387$, $P=.035$), however, no significant correlation between CPZeq and the other task performances or each NIRS measurement was found in the first group. No significant

Table 2
Comparisons in frontal tasks and NIRS measurements between patients with schizophrenia and controls.

a. First group

	Schizophrenia	Control	*df*	*t*	*P* value	C.C.	Effect size
	Mean (SD)	Mean (SD)				(%)	(d)
Task performance							
VFT letter	13 (3.9)	17.4 (3.8)	58	4.38	<.001	71.7	0.988
VFT category	23.7 (5.0)	28.9 (4.6)	57.5	4.22	<.001	73.3	0.962
TOH4	6.1 (3.1)	11 (3.6)	56.8	5.65	<.001	73.3	1.181
Sternberg	20.8 (2.8)	22.6 (1.7)	48.8	2.91	.005	66.7	0.707
Stroop	37.8 (4.0)	39.6 (0.67)	30.6	2.42	<.001	61.7	0.601
⊿[Oxy-Hb] (μmol/L)							
VFTletter	0.69 (0.86)	1.56 (1.01)	56.7	3.62	.001	70	0.851
VFTcategory	0.46 (0.68)	1.07 (0.85)	55.3	3.08	.003	70	0.744
TOH4	0.78 (0.78)	1.77 (1.18)	50.2	3.86	<.001	73.3	0.897
Sternberg	0.31 (0.72)	0.80 (0.87)	56	2.37	.021	66.7	0.589
Stroop	0.30 (0.87)	0.41 (0.95)	58	0.5	.621	50	0.129

b. Second group

	Schizophrenia	Control	*df*	*t*	*P* value	C.C.	Effect size
	Mean (SD)	Mean (SD)				(%)	(d)
Task performance							
VFT letter	14.0 (4.7)	17.5 (4.8)	58	2.84	.006	65	0.692
VFT category	22.1 (5.9)	28.1 (4.4)	53.8	4.41	<.001	71.7	0.993
TOH4	9.7 (5.9)	13.9 (6.5)	57.4	2.63	.011	60	0.646
Sternberg	21.1 (3.5)	22.4 (2.0)	58	1.67	.102	60	0.425
Stroop	38.1 (3.5)	39.1 (2.9)	58	1.2	.237	60	0.307
⊿[Oxy-Hb] (μmol/L)							
VFTletter	0.15 (0.57)	0.73 (0.78)	52.9	3.28	.002	61.7	0.784
VFTcategory	0.34 (0.62)	0.82 (0.82)	54	2.56	.013	61.7	0.633
TOH4	0.56 (0.43)	1.17 (0.79)	45	3.73	<.001	63.3	0.873
Sternberg	−0.12(0.79)	0.36 (1.1)	58	1.98	.053	45	0.499
Stroop	−0.02(0.77)	0.27 (0.91)	58	1.33	.188	51.7	0.342

Abbreviation: C.C., correct classification.