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The *p250GAP* Gene Is Associated with Risk for Schizophrenia and Schizotypal Personality Traits

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Abstract

Background: Hypofunction of the glutamate *N*-Methyl-d-aspartate (NMDA) receptor has been implicated in the pathophysiology of schizophrenia. p250GAP is a brain-enriched NMDA receptor-interacting RhoGAP. p250GAP is involved in spine morphology, and spine morphology has been shown to be altered in the post-mortem brains of patients with schizophrenia. Schizotypal personality disorder has a strong familial relationship with schizophrenia. Several susceptibility genes for schizophrenia have been related to schizotypal traits.

Methods: We first investigated the association of eight linkage disequilibrium-tagging single-nucleotide polymorphisms (SNPs) that cover the *p250GAP* gene with schizophrenia in a Japanese sample of 431 schizophrenia patients and 572 controls. We then investigated the impact of the risk genetic variant in the *p250GAP* gene on schizotypal personality traits in 180 healthy subjects using the Schizotypal Personality Questionnaire.

Results: We found a significant difference in genotype frequency between the patients and the controls in rs2298599 ($\chi^2 = 17.6$, $p = 0.00015$). The minor A/A genotype frequency of rs2298599 was higher in the patients (18%) than in the controls (9%) ($\chi^2 = 15.5$, $p = 0.000083$). Moreover, we found that subjects with the rs2298599 risk A/A genotype, compared with G allele carriers, had higher scores of schizotypal traits ($F_{1,178} = 4.08$, $p = 0.045$), particularly the interpersonal factor ($F_{1,178} = 5.85$, $p = 0.017$).

Discussion: These results suggest that a genetic variation in the *p250GAP* gene might increase susceptibility not only for schizophrenia but also for schizotypal personality traits. We concluded that the *p250GAP* gene might be a new candidate gene for susceptibility to schizophrenia.

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Introduction

Schizophrenia 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 and have estimated the rate of schizophrenia heritability at 80% [1,2]. Although genes implicated in the pathogenesis of schizophrenia have been found using several approaches, such as through association studies of candidate genes, genome-wide association studies (GWAS), copy number variation (CNV) studies and pedigree studies [3,4], the exact genetic factors of this complex disease remain to be explained.

Hypofunction of the glutamate *N*-Methyl-d-aspartate (NMDA) receptor is strongly implicated in the pathophysiology of schizophrenia. NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, mimic symptoms of the disorder in humans and exacerbate symptoms in patients with schizophrenia [5]. These NMDA receptor antagonists induce schizophrenia-like symptoms in humans. Preclinically, they have been shown to

induce similar symptoms and to induce neural circuitry changes reminiscent of schizophrenia [6]. The ability of these NMDA receptor antagonists to induce a schizophrenia-like phenotype supports the concept that schizophrenia may be the result of reduced or abnormal functioning of NMDA receptors. Altered NMDA receptor binding density in several brain regions, such as in the anterior cingulate cortex, has been reported in schizophrenia [7,8]. The NR2 subunits of the NMDA receptor are spatially and developmentally regulated, and they provide an important level of receptor regulation [9,10]. NR2A and NR2B are the predominant subunits in the cortex, striatum and hippocampus [11,12,13]. In particular, these three areas are closely associated with the pathology of schizophrenia and with the neural circuits within and between these regions [14]. In patients with schizophrenia, alterations have been observed in the NR2 subunit mRNA and protein in the prefrontal cortex, including a reduction in NR2A mRNA and NR2B protein levels [15,16]. Additionally, the NR2B subunit mRNA levels were increased in the hippocampus [17]. Therefore, different expression of NR2 subunits could play an important role in the pathophysiology of schizophrenia.

The NMDA receptor regulates activity-dependent spine morphological plasticity by modulating the actin cytoskeleton [18]. As the key regulators of actin cytoskeleton dynamics, the Rho family of GTPases, including RhoA, Cdc42, and Rac1 and their regulators, play an important role in NMDA receptor-mediated spine morphogenesis [18,19]. In our previous study, we identified the *p250GAP* gene (also known as *p200RHOGAP*, *GRIT*, *KIAA0712*, *RICS*, or *ARHGAP32*: OMIM 608541) as a novel NMDA receptor-interacting RhoGAP [20,21,22,23]. This gene spans approximately 56.17 kb of the genomic DNA and is located on chromosome 11q24.3. p250GAP is highly enriched in the central nervous system, is concentrated in the post-synaptic densities in neurons and is co-localized with the NR2B subunit of the NMDA receptor [20]. Knockdown of p250GAP increased spine width and elevated the endogenous RhoA activity in primary hippocampal neurons, suggesting that p250GAP regulates spine morphogenesis through its RhoGAP activity for RhoA [24]. Importantly, p250GAP activity and localization within neurons are regulated by NMDA receptor activity [20,24], suggesting that p250GAP, together with the NMDA receptor, regulates NMDA receptor-mediated spine morphogenesis. Given that neuropathological studies of schizophrenia have shown alterations in spine morphology [25,26], we hypothesized that the *p250GAP* gene may be related to the pathophysiology of schizophrenia. In this study, we investigated the association between the *p250GAP* gene and schizophrenia in a Japanese population using a gene-based approach.

Schizotypal personality disorder (SPD) is one of the schizophrenia spectrum disorders and is characterized by social avoidance, ideas of reference, vagueness, magical thinking, odd speech, illusions and paranoid ideation. The lifetime prevalence of SPD has been estimated at 3.9% [27], making it one of the more common psychiatric disorders. The prevalence rate of SPD in relatives of individuals with schizophrenia (6.9%) was higher than the prevalence rates found either in relatives of individuals with other psychiatric disorders or in mentally healthy subjects [28]. Twin studies have estimated that the heritability of the latent liability to SPD is 61–72% [29,30]. Premorbid SPD is related to the development of schizophrenia [31]. These findings suggest that SPD shares common genetic influences with schizophrenia. The traits of SPD were incorporated in the SPD criteria in the *Diagnostic and Statistical Manual of Mental Disorders*, third edition (DSM-III), and the traits are listed in the DSM-IV-TR on Axis II. These traits can be identified using a well-validated questionnaire, such as the Schizotypal Personality Questionnaire (SPQ) [32]. The heritability rates of three schizotypal trait factors, cognitive/perceptual, interpersonal and disorganization, have been estimated at 40 to 60% [33,34]. We recently demonstrated that a genome-wide genetic variant for schizophrenia in the *ZNF804A* gene was associated with schizotypal personality traits [35]. Additionally, we investigated whether a genetic variant in the *p250GAP* gene was associated with schizotypal personality traits in healthy subjects.

Materials and Methods

Ethics statement

Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was performed in accordance with the World Medical Association's Declaration of Helsinki and approved by the Osaka University Research Ethics Committee.

Subjects

The subjects of our genetic association study were 431 patients with schizophrenia (48.7% male (210 males, 221 females), mean age ± SD was 49.7±15.4 years) and 572 healthy controls (46.7% male (267 males, 305 females), mean age ± SD was 61.9±20.4 years). The sex ratio did not differ significantly between the groups ($\chi^2 = 0.41$, $p = 0.52$), but the mean age was significantly different ($z = -11.49$, $p < 0.001$). The subjects were all biologically unrelated and were Japanese. The subjects were recruited from both outpatient and inpatient units at Osaka University Hospital and other psychiatric hospitals. Each patient with schizophrenia had been diagnosed by at least two trained psychiatrists by unstructured clinical interviews, according to the criteria of the DSM-IV. When the diagnosis of the two trained psychiatrists was discordant, they discussed the diagnosis. When the diagnostic disputes were resolved and the patient was diagnosed as schizophrenic, we included the patient. When the diagnostic disputes were not resolved by discussion or the patient was not diagnosed as schizophrenia, we excluded the patient. Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals with current or past contact with psychiatric services, with experience with psychiatric medications or who were not Japanese. We did not assess the controls for their family history of mental disorders, such as schizophrenia, bipolar disorder, or major depressive disorder. The ethnicity was determined by self-report and was not confirmed by genetic analyses.

Data for the schizotypal personality trait analysis were available for 180 healthy subjects [48.3% male (87/93), mean age ± SD: 36.5±11.5 years]. The subjects were included in the genetic association analysis. The subjects included in the analysis met additional criteria. Psychiatrically, medically and neurologically healthy controls were evaluated using the Structured Clinical Interview for DSM-IV-Non-Patient Edition (SCID-I/NP) to exclude individuals who had received psychiatric medications or who had first- or second-degree relatives with psychiatric disorders. Additionally, subjects were excluded from this study if they had neurological or medical conditions that could have potentially affected their central nervous system, such as atypical headaches, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation.

SNP selection, genotyping and genomic sequencing

This study was designed to examine the association between the *p250GAP* gene and schizophrenia by tagging single-nucleotide

polymorphisms (SNPs) in the *p250GAP* gene and its flanking regions (±5 kb). Of the 31 SNPs in the *p250GAP* gene and flanking regions, we selected eight tagging SNPs using the TAGGER algorithm (Paul de Bakker, http://www.broad.mit.edu/mpg/tagger) with the criteria of r^2 greater than 0.5 in 'pairwise tagging only' mode and a minor allele frequency (MAF) greater than 5%. The selection was implemented in Haploview 4.2 using HapMap data release 24/PhaseII Nov 08, on NCBI B36 assembly, dbSNP b126 (Japanese in Tokyo (JPT), Chr 11: 128,338,052..128,404,222) (Table S1). The eight tagging SNPs were rs493172, rs10893947, rs2276027, rs3796668, rs581258, rs3740829, rs546239 and rs2298599. The markers are shown in Table 1; the orientation and the alleles are reported on the genomic minus strand. The positions of the eight SNPs analyzed in the present study and the LD relationships between the SNPs in a HapMap JPT population are shown in Figure 1. Venous blood was collected from the subjects. Genomic DNA was extracted from the whole blood using standard procedures. The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems; Foster City, California, USA) as previously described [36,37]. Detailed information on the PCR conditions is available upon request. Genotyping call rates were 99.3% (rs493172), 98.9% (rs10893947), 99.1% (rs2276027), 99.7% (rs3796668), 98.4% (rs581258), 99.2% (rs3740829), 98.5% (rs546239) and 99.3% (rs2298599). No deviations from the Hardy-Weinberg equilibrium (HWE) in the examined SNPs were detected ($p>0.05$). Additionally, with 48 subjects with schizophrenia, we confirmed a SNP significantly associated with schizophrenia, genotyped by the TaqMan method, using direct DNA sequencing. These subjects were included in the genetic association analysis. The genomic regions were amplified by PCR using a pair of primers for rs2298599, 5′- AAGTCAGCCCA-GACTCTCCA -3′ and 5′- GAGGGAGGAAGGGATTTTTG -3′. PCR for each sample was carried out in a total volume of 40 μl using a Gene Amp® PCR System 9700 (Applied Biosystems, CA, U.S.A). The PCR cycling conditions were 94°C for 10 minutes, 30 cycles at 94°C for 1 minute, 60°C for one minute and 72°C for 1 minute, followed by an incubation at 72°C for 10 minutes. The PCR products were purified using a QIA quick® PCR Purification Kit (QIAGEN, CA, USA), and the purification products were sequenced using a Big Dye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Cycle sequencing conditions were 96°C for 2 minutes, 25 cycles of 96°C for 20 seconds, 50°C for 30 seconds and 60°C for 2 minutes, using a Gene Amp® PCR System 9700. The PCR products from the cycle sequencing were purified using a Big Dye® XTerminator™ Purification Kit (Applied Biosystems, CA, U.S.A.), and they were sequenced using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, CA, U.S.A.). The sequencing was checked with SEQUENCHER ver. 4.7 (Gene Codes, U.S.A.).

Schizotypal personality trait analysis

To assess schizotypal personality traits, a full Japanese version of the SPQ was administered to healthy subjects [38,39]. The SPQ is a 74-item self-report questionnaire with a "yes/no" response format [40]. All items answered "yes" were scored 1. The SPQ measures nine subscales of specific schizotypal features, which are ideas of reference, odd beliefs/magical thinking, unusual perceptual experiences, suspiciousness/paranoid ideation, social anxiety, no close friends, constricted affect, eccentric/odd behavior and odd speech. The total SPQ score was obtained by summing the scores from all of the items. The three schizotypal trait factors, cognitive/perceptual, interpersonal and disorganization, were derived by summing the related subscale raw scores according to the three-factor model of Raine and colleagues [32]. Full-scale IQ was assessed using the Wechsler Adult Intelligence Scale, Revised or Third edition.

Statistical analysis

Differences in clinical characteristics between the patients and the controls or between the genotype groups were analyzed using the χ^2 test for categorical variables and the Mann-Whitney *U*-test for continuous variables, using the PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS [41]). The power estimates were based on the allele frequency of 0.35 (rs2298599) in the controls and an alpha level of 0.05. Power was calculated under a prevalence of 0.01 using a multiplicative model that assumed varying degrees of the odds ratio (OR). Statistical analyses for the genetic associations were performed using the SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan). Deviation from the HWE was tested using χ^2 tests for goodness of

Table 1. Genotypic and allelic distributions for SNPs in the *p250GAP* between patients with schizophrenia and controls.

Marker				SCZ ($n=431$)			CON ($n=572$)			Genotypic	SCZ	CON	Allelic	OR
SNP IDs	Position[a]	M/m	gene	M/M	M/m	m/m	M/M	M/m	m/m	p ($\chi 2$)	MAF		p ($\chi 2$)	(95% CI)
rs493172	128388089	C/G	intron1	346	77	3	451	116	3	0.63 (0.9)	0.10	0.11	0.49 (0.5)	0.90 (0.67–1.21)
rs10893947	128375634	G/A	intron1	122	217	88	177	294	94	0.25 (2.8)	0.46	0.43	0.14 (2.2)	1.15 (0.96–1.37)
rs2276027	128355514	T/C	intron8	241	158	27	303	229	36	0.57 (1.1)	0.25	0.27	0.42 (0.7)	0.92 (0.75–1.13)
rs3796668	128349062	A/C	intron11	186	182	62	206	292	72	**0.020 (7.8)**	0.36	0.38	0.22 (1.5)	0.89 (0.74–1.07)
rs581258	128348083	A/G	exon12	293	125	8	373	171	17	0.46 (1.6)	0.17	0.18	0.32 (1.0)	0.89 (0.70–1.12)
rs3740829	128344366	A/G	exon13	375	50	2	513	54	1	0.37 (2.0)	0.06	0.05	0.18 (1.8)	1.30 (0.89–1.91)
rs546239	128340968	A/G	3′	325	91	9	402	149	12	0.18 (3.4)	0.13	0.15	0.11 (2.6)	0.81 (0.63–1.05)
rs2298599	128340162	G/A	3′	167	184	76	219	296	53	**0.00015 (17.6)**	0.39	0.35	0.07 (3.3)	1.18 (0.99–1.42)

SCZ: patients with schizophrenia, CON: controls, M: major allele, m: minor allele, MAF: minor allele frequency, OR: odds ratio, 95%CI: 95% confidence interval.
[a]db SNP build 129.
All of the alleles are represented according to the minus strand DNA sequence. Numbers of genotypes were represented as genotype counts. *P* values<0.05 are in boldface and underlined.
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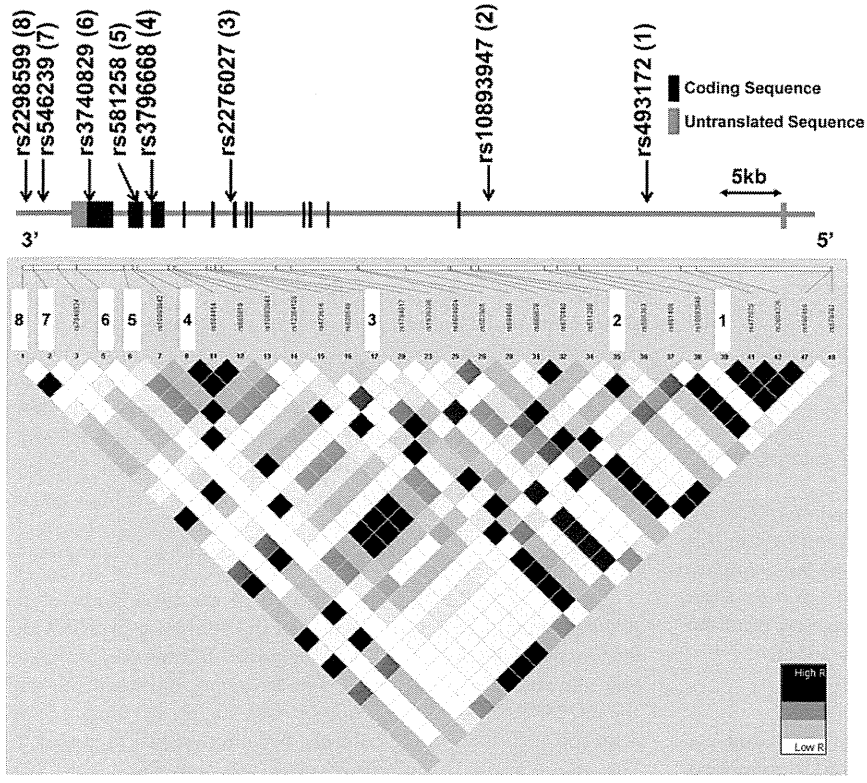

Figure 1. The genomic structure of *p250GAP* and linkage disequilibrium of the *p250GAP* in the HapMap JPT. The genomic structure of *p250GAP* is based on an entry in the Entrez Gene database (National Center for Biotechnology Information). The locations of the SNPs analyzed in this study are indicated by arrows. The numbers indicated in parentheses refer to the numbering of the SNPs in the linkage disequilibrium (LD) diagram. The distances of the exons-introns and the intermarkers are drawn to scale. The LDs between the pairwise SNPs are shown using the r^2 value at the bottom of the map of the gene structure for the HapMap JPT samples. High levels of LD are represented by black (r^2) coloring, with increasing color intensity shown by the color bars.
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fit. The allelic and genotypic distributions of *p250GAP* polymorphisms between the patients and the controls were analyzed using χ^2 tests.

Pairwise linkage disequilibrium (LD) analyses, expressed by r^2, were applied to detect the intermarker relationships in each group using the Haploview 4.2 software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype frequencies were estimated using the maximum likelihood method with the genotyping data. We used the expectation-maximization algorithm from the SNPAlyze V5.1.1 Pro software. Rare haplotypes, detected in less than 3% of the patients and the controls, were excluded from the haplotypic association analysis, as previously described [42,43]. Using a 2×2 contingency table approach, we performed 10,000 permutations of significance tests to determine empirical significance. We used a 2- to 8-window fashion analysis. We applied Bonferroni corrections in allelic and genotypic association analyses (eight tests) and in haplotypic association analyses (28 independent global tests).

The effects of the *p250GAP* genotype on the total score and on the three factors of the SPQ were analyzed by a one-way analysis of variance (ANOVA). To control confounding factors, the effect of the *p250GAP* genotype on the significance factor of the SPQ was analyzed by a one-way analysis of covariance (ANCOVA). Age, sex and education years were used as covariates because the SPQ total score and the three factors were correlated with these confounding factors in a previous study [44]. Standardized effect sizes were calculated using Cohen's *d* method (http://www.uccs.edu/faculty/lbecker). All *p* values are two tailed, and statistical significance was defined as $p<0.05$.

Results

Genetic association analysis

Our study size of 431 patients with schizophrenia and 572 controls had sufficient power (>80%) to detect a genetic effect at ORs of 1.30 or larger when the allele frequency was 0.35. The genotype and allele frequencies of the eight tagging SNPs located in the *p250GAP* gene and flanking regions are summarized in Table 1. We found significant differences in genotype frequencies between the patients and the controls in rs3796668 ($\chi^2=7.8$, $p=0.020$) and rs2298599 ($\chi^2=17.6$, $p=0.00015$). No allelic or genotypic associations were observed with schizophrenia for any other SNPs ($p>0.05$). The major genotype frequency of rs3796668 was significantly higher in the patients with schizophrenia (43%) than in the controls (36%) ($\chi^2=5.2$, $p=0.023$), but no differences were observed in the frequencies of the minor or heterozygous genotypes of rs3796668 ($p>0.05$). The minor genotype frequency of rs2298599 was higher in the patients with schizophrenia (18%) than in the controls (9%) ($\chi^2=15.5$, $p=0.000083$), but no differences were observed in the frequencies of the major or heterozygous genotypes of rs2298599 ($p>0.05$). The evidence for genotypic association of rs2298599 remained significant after a Bonferroni correction for multiple tests (corrected $p=0.0012$). Genomic sequencing data for rs2298599 for each individual were in agreement with genotyping data using the TaqMan methods. Haplotype analysis showed a marginally significant association with schizophrenia in the rs3740829- rs546239- rs2298599 haplotype ($\chi^2=7.9$, global $p=0.049$) (Table S2). However, the

Table 2. Demographic variables for subjects included in the SPQ analysis.

Variables	Total (n=180)	G carrier (n=159)	A/A (n=21)	p values	(z)
Age (years)	36.6±11.5	36.5±11.5	37.5±11.8	0.69	0.40
Sex (male/female)[a]	87/93	77/82	10/11	0.94	<0.01
Education (years)	15.4±2.4	15.6±2.4	14.4±2.0	**0.041**	−2.05
Full scale IQ	109.0±12.0	109.0±12.2	108.8±11.2	0.75	−0.33

Means ± SD are shown. P values<0.05 are in boldface and underlined.
[a]χ^2 test.
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association did not survive correction for multiple testing (p>0.05 after Bonferroni correction).

The LD relationships between the investigated markers are provided in Figure S1. The LD pattern observed in our controls was similar to our patients and the JPT HapMap samples; however, it was different from the LD pattern of the Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) HapMap samples.

In silico genotype-expression analysis

We examined an association between the rs2298599 and the expression levels of the *p250GAP* gene in immortalized lymphoblasts derived from 45 HapMap JPT subjects using WGAViewer software (http://compute1.lsrc.duke.edu/softwares/WGAViewer). However, *in silico* analysis revealed that there was no significant association between the SNP and the *p250GAP* expression in the immortalized lymphoblasts (p = 0.28).

Impact of *p250GAP* genotype on schizotypal personality traits

We examined a possible association between the *p250GAP* genotype of rs2298599 and schizotypal personality traits in healthy subjects. Compared to controls, patients with schizophrenia were significantly more likely to carry the rs2298599 A/A genotype. Therefore, these analyses focused on a comparison of homozygous risk A/A genotype carriers versus homozygous carriers of one or two copies of the G allele (a combined G/G and G/A genotype group), under a recessive inheritance model of the risk A/A genotype. Demographic variables, age, sex, and full-scale IQ were not significantly different between the genotype groups, except for years of education (z = −2.05, p = 0.041) (Table 2). We first examined the possible effect of *p250GAP* rs2298599 on the total SPQ score and found a significant effect of the genotype ($F_{1,178}$ = 4.08, p = 0.045) (Table 3). Then, we investigated the genotype effects on the three SPQ factors, cognitive/perceptual, interpersonal and disorganization. A significant genotype effect was observed on the interpersonal factor ($F_{1,178}$ = 5.85, p = 0.017), but no significant genotype effects were observed on the cognitive/perceptual or disorganization factors (p>0.1). The effect of genotype on the interpersonal factor remained significant after adjusting for confounding factors ($F_{1,175}$ = 4.71, p = 0.031). Subjects with the risk A/A genotype of rs2298599 showed higher scores on schizotypal traits, particularly the interpersonal factor, compared with subjects with the G allele (Figure 2). The effect sizes of the total score and interpersonal factor were 0.41 and 0.47, respectively. When the two genotypes were divided into opposite two genotype groups (homozygous carriers of one or two copies of the A allele versus homozygous G/G genotype carriers) under a dominant model of inheritance, there was no significant difference in scores between A carriers and individuals with G/G genotype (p>0.05, Table S3).

Discussion

This study is the first investigation of the association of the *p250GAP* gene with schizophrenia. In this study, we first provided evidence that a genetic variant of the *p250GAP* gene was associated with the risk for schizophrenia. The frequency of individuals with the rs2298599 risk A/A genotype was higher in patients with schizophrenia than in the controls. Second, we indicated that the risk genotype of the *p250GAP* gene was associated with high schizotypal personality traits, particularly the interpersonal factor, in healthy subjects. Individuals with the rs2298599 risk A/A genotype scored higher on schizotypal personality traits and the interpersonal factor than did individuals with non-risk genotypes. These findings suggest that the *p250GAP* gene may be related to the risk for schizophrenia and the schizotypal personality traits.

Rs2298599 is situated within the relatively large LD block, which includes the *p250GAP* and the *P53AIP1* (OMIM 605426) genes (Figure S2). The SNP is located 2.9 kb downstream of the *p250GAP* gene and located 22.1 kb upstream of the *P53AIP1* gene. To confirm whether a significant association signal of rs2298599 with schizophrenia is attributed to the *p250GAP* gene, we checked strength of LDs in the genomic region (±50 kb) around rs2298599 using HapMap data (JPT, Chr 11: 128,290,162..128,390,161). Seven SNPs was related to rs2298599 with the criteria of r^2 greater than 0.8. Of the seven SNPs, five SNPs were located 5′ upstream from rs2298599 and four SNPs were included in the *p250GAP* gene. Two SNPs were located 5.2 and 7.9 kb downstream from rs2298599. These findings suggest that our association signal could be attributed to the *p250GAP* but not the *P53AIP1* gene. However, the *P53AIP1* may be a susceptibility gene for schizophrenia. Future

Table 3. Association of the *p250GAP* gene risk variant with the schizotypal personality traits.

SPQ	Total	G carrier	A/A		Genotype effect		
Variables	(n=180)	(n=159)	(n=21)	Cohen's d	$F_{1,178}$	p values	η^2
Total score	10.7±8.9	10.3±8.4	14.4±11.4	−0.41	4.08	**0.045**	0.02
Cognitive/perceptual	3.3±3.8	3.2±3.7	4.0±4.5	−0.19	0.94	0.33	0.01
Interpersonal	5.0±4.5	4.7±4.1	7.2±6.3	−0.47	5.85	**0.017**	0.03
Disorganization	3.1±3.3	3.0±3.3	4.1±3.8	−0.31	2.13	0.15	0.01

SPQ: Schizotypal Personality Questionnaire. Means ± SD are shown. The effect sizes are typically categorized as small (d = 0.20, η^2 = 0.01), medium (d = 0.50, η^2 = 0.06) or large (d = 0.80, η^2 = 0.14). Significant p values are shown in boldface and underlined.
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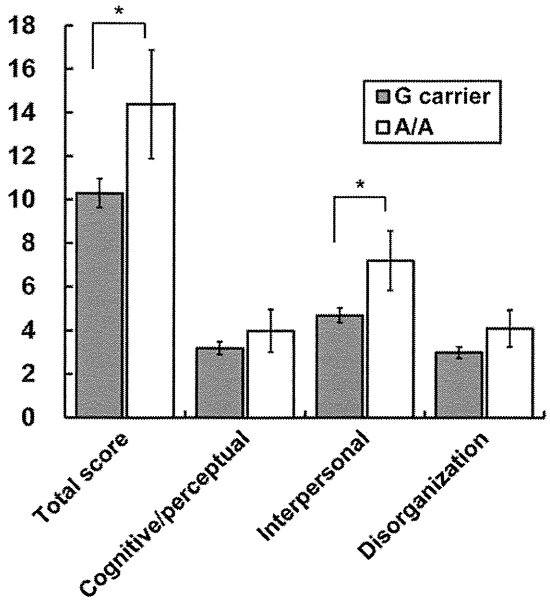

Figure 2. The association between the risk-associated *p250GAP* genotype and SPQ total score and the three factors. The gray bars represent individuals who are G-carriers (G/G and G/A genotypes) of rs2298599. The white bars represent individuals with the A/A genotype of the SNP. Error bars represent standard errors of the mean. * $p<0.05$.
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studies are required to investigate the association between the *P53AIP1* and schizophrenia.

Although significant associations between the *p250GAP* gene and schizophrenia were observed in this study, no experimental evidence has indicated that the rs2298599 SNP of *p250GAP* is functional. To define a possibly functional SNP associated with the disease, followed by evaluation of altered function caused by the relevant SNP, may be able to narrow down the region of the association observed with the rs2298599. For example, gene expression analyses at either mRNA or protein levels of the *p250GAP* gene using postmortem or lymphoblast samples may be an alternative approach. We examined an association between the rs2298599 and *p250GAP* expression in immortalized lymphoblasts. However, *in silico* analysis revealed that the SNP might not be related to *p250GAP* mRNA expression in a Japanese population. A future biological study of the function of rs2298599 or *p250GAP* gene is required to verify our results.

MicroRNAs (miRs) regulate cellular fate by controlling the stability or translation of the mRNA transcripts. The miR132, located on 17p12.3, controls p250GAP protein levels and regulates neuronal morphogenesis by decreasing the levels of p250GAP [45]. The miR132 target sequence in the *p250GAP* 3′UTR, AACAGTCCACTGTCCAGCAGAGG, is conserved across vertebrate evolution. We performed a mutation search of the genomic region (the target sequence of miR132±250 bp) in the *p250GAP* gene using 48 patients with schizophrenia to evaluate the presence of a genetic variant in this region. However, we had no polymorphisms in our sequence data. This result suggests that the miR132 target sequence in the *p250GAP* might not play a major role in risk for schizophrenia.

Several molecular genetic studies have investigated the influences of susceptibility genes for schizophrenia on schizotypal personality traits. These studies have reported associations between the *COMT* [46,47,48], *NRG1* [49], *DTNBP1* [50,51], *RGS4* [52], *DAAO* [51] and *ZNF804A* [35] genes and schizotypal components. Risk alleles or haplotypes of schizophrenia were correlated with high scores on schizotypal personality traits. Of these genes, the *COMT*, *NRG1*, *DTNBP1*, *DAAO* and *RGS4* genes, as well as the *p250GAP* gene, are directly or indirectly responsible for NMDA receptor-mediated glutamate transmission or signaling via glutamate receptors [53]. However, involvement of the glutamate NMDA receptors in SPD is still unknown. Further research will need to clarify the relationship between the glutamate NMDA receptors and SPD.

The interpretation of our results has several limitations. We found a significant association of the *p250GAP* gene with schizophrenia using 431 patients with schizophrenia and 572 controls. Our sample sizes had sufficient power (>0.80) to detect the effects of ORs of 1.30 or larger. Because our results were based on a relatively small sample to detect the effects of ORs of 1.30 or fewer, a future replication study using larger sample sizes is needed to confirm our findings. Our positive results might have been derived from a sample bias due to population stratification and non-age-matched samples, although the Japanese are a relatively homogeneous population. We used schizotypal personality traits as a phenotype of interest. As the assessment of the personality traits was based on a self-reported questionnaire, it was not an objective measurement. Importantly, to be included in the SPQ analysis, subjects were not required to meet criteria for SPD. We had hypothesized that schizotypal personality trait is a continuous measure of the genetic liability to schizophrenia. G allele carriers had marginally higher years of education and lower scores on schizotypal traits than did subjects with the risk A/A genotype. In a previous study, years of education had significant inverse effects on the total SPQ score and the three factor scores, indicating that the SPQ scores decreased with increased years of education [44]. The educational level difference between the genotype groups may have affected the genotype effects on the schizotypal personality trait. However, our results remained significant after adjusting for years of education.

In this study, we proposed *p250GAP* as a new candidate gene for susceptibility to schizophrenia. The association between the *p250GAP* gene and schizophrenia might partially explain the relationship between the hypofunction of the glutamate NMDA receptor and schizophrenia. Future studies are required to confirm the association between the *p250GAP* gene and schizophrenia in other populations.

Supporting Information

Figure S1 Linkage disequilibrium pattern of eight SNPs in the patient, control, HapMap JPT and CEU groups. The linkage disequilibriums (LDs) between the pairwise SNPs are shown using the r^2 value separately for the patients with schizophrenia, the controls, the HapMap JPT samples and the HapMap CEU samples. High levels of LD (r^2) are represented by black coloring, and increasing color intensity from 0 to 100 is shown by the color bars. The numbers (from 1 to 8) in the boxes refer to the eight tagging SNPs; rs493172 (1), rs10893947 (2), rs2276027 (3), rs3796668 (4), rs581258 (5), rs3740829 (6), rs546239 (7) and rs2298599 (8).
(TIF)

Figure S2 Linkage disequilibrium in the genomic region (±50 kb) around rs2298599 SNP in HapMap JPT. LD structure is based on an entry in the HapMap data release 24/PhaseII Nov 08, on NCBI B36 assembly, dbSNP b126 (JPT, Chr 11: 128,290,162..128,390,161). The LD structure between the pairwise SNPs is shown using the r^2 value. High levels of LD are represented by black (r^2) coloring, with increasing color intensity.
(TIF)

Table S1 Selected tagging SNPs in the *p250GAP* gene and its flanking regions.
(DOC)

Table S2 Haplotype analysis of the *p250GAP* gene between patients with schizophrenia and the controls.
(DOC)

Table S3 Association of the *p250GAP* gene variant with schizotypal personality traits under dominant model of inheritance.
(DOC)

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Author Contributions

Conceived and designed the experiments: KO RH TN TY MK MT. Performed the experiments: TO YY HY MF SU. Analyzed the data: KO RH TN MI HK. Contributed reagents/materials/analysis tools: TO YY HY MF SU MI HK TY MK MT. Wrote the paper: KO RH TN.

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Frontal and right temporal activations correlate negatively with depression severity during verbal fluency task: A multi-channel near-infrared spectroscopy study

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ABSTRACT

Multi-channel near-infrared spectroscopy (NIRS) is a noninvasive, on-the-spot, functional neuroimaging technique allowing detection of the spatiotemporal characteristics of brain activity. Previous NIRS studies indicated the oxy-hemoglobin (oxy-Hb) increase during a verbal fluency task (VFT) is attenuated in patients with major depressive disorder (MDD) as compared with healthy controls. However, the possible relationship between depression symptom severity and oxy-Hb change on NIRS has not yet been elucidated. To examine this relationship, we recruited 30 patients with MDD and 30 age-, gender- and intelligence quotient-matched controls. All underwent NIRS during VFT. As expected, the oxy-Hb increase during the task was significantly smaller in patients than in controls. After false discovery rate correction using 31 channels, the mean increase in oxy-Hb during the task showed a significant negative correlation with the total score of the Hamilton Rating Scale for Depression 21-item version (ch25: rho = −.56; FDR-corrected *p*: .001). When each item of the HAM-D21 was examined individually, insomnia early in 9 channels (rho = −.63 to −.46; FDR corrected *p*: .000–.014), work and activity in 2 channels (rho = −.61 to −.57; FDR corrected *p*: .001 to .003) and psychomotor retardation in 12 channels (rho = −.70 to −.44; FDR corrected *p*: .000–.018) showed significant negative correlations with the mean oxy-Hb increase in the right frontal temporal region. Although it is possible that our results were affected by medication, these data suggest reduced right frontal temporal activation on NIRS during VFT is related to the symptom severity of MDD.

1. Introduction

Major depressive disorder (MDD) is a severe and common psychiatric disorder with a lifetime prevalence of 6.7 per 100 (Waraich et al., 2004). Although depressive symptoms per se do not specifically appear in MDD but also in other psychiatric disorders including bipolar disorders, we do not have an objective diagnostic marker to obtain a clear-cut diagnosis for those patients. In Japan, a relatively new neuroimaging method, near-infrared spectroscopy (NIRS) has been approved by the Ministry of Health, Labor and Welfare as a highly advanced medical technology to help distinguish between schizophrenia, depression and bipolar disorders in 2009. Verbal fluency task (VFT) is recommended as an activation task because of a relatively rich store of data. VFT is an easy task to examine the executive function and frequently used in neuroimaging studies (Alvarez and Emory, 2006) and is known to activate prefrontal cortex (PFC) in healthy subjects (Frith et al., 1991; Schlösser et al., 1998). Numerous neuropsychological studies suggest that patients with MDD show executive dysfunction (Gohier et al., 2009; Rose and Ebmeier, 2006; Fossati et al., 2003; Porter et al., 2003; Degl'Innocenti et al., 1998).

Multi-channel near-infrared spectroscopy (NIRS) is a noninvasive, on-the-spot, restraint-free functional neuroimaging technique allowing detection of the spatiotemporal characteristics of brain

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function near the brain surface using near-infrared light (Strangman et al., 2002a; Boas et al., 2004). NIRS has enabled bedside measurement of the concentrations of oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) changes with a high time resolution (.1 s). The concentrations of oxy-Hb and deoxy-Hb are assumed to reflect the regional cerebral blood volume (rCBV) changes, which was supported by the simultaneous NIRS and PET study (Villringer et al., 1997; Ohmae et al., 2006).

In fact, numerous studies have demonstrated that the oxy-Hb increase in the fronto-temporal regions during a VFT is significantly smaller in patients with MDD than in those with bipolar disorder or healthy controls (Pu et al., 2008; Kameyama et al., 2006; Suto et al., 2004; Matsuo et al., 2002). Moreover, NIRS studies using VFT have also demonstrated frontal lobe dysfunction in schizophrenia (Suto et al., 2004; Takizawa et al., 2008), and panic disorder (Nishimura et al., 2007). However, the relationship between depression symptom severity at the time of examination and oxy-Hb change on NIRS has not yet been clarified.

In neuroimaging studies using other methodologies, focusing on cortex level that NIRS reflects, positron emission tomography (PET) studies found that abnormal reductions of cerebral blood flow (CBF) and metabolism in patients with MDD in PFC (Kimbrell et al., 2002; Bench et al., 1995; Mayberg et al., 1994; Baxter et al., 1989). As for the relationship between executive function and CBF or metabolism, Elliott et al. (1997) showed activation in PFC was significantly attenuated relative to controls during the Tower of London planning task in PET study. In a functional magnetic resonance imaging (fMRI) study, depressed patients showed significant decreased prefrontal activation during VFT (Okada et al., 2003).

As for the relationship between depression symptom severity and frontal lobe function, Brody et al. (1999) found a positive correlation between change in Hamilton Rating Scale for Depression (HAM-D) scores and change in normalized inferior frontal gyrus (IFG) and ventrolateral PFC (VLPFC) metabolism, which indicates that IFG metabolism increased and VLPFC metabolism decreased as depression symptoms became better. Other initial studies also suggest that abnormal functions in dorsolateral PFC (DLPFC) are mood state dependent, attenuated during the depressed mood and reversing during symptom remission (Bench et al., 1995; Mayberg et al., 1994). In contrast, Drevets et al. (2002) showed the persistence of abnormal metabolic deficits using PET measures in the dorsomedial/dorsal anterolateral PFC in MDD during treatment. According to a review by Drevets (2000), a complex relationship exists between depression symptom severity and metabolic activity in the orbital cortex and VLPFC.

Findings obtained by more recent studies investigating cross-sectional relationship between depression symptom severity and brain function assessed by basal regional CBF and metabolism are also inconsistent. For example, Périco et al. (2005) reported that depression symptom severity was negatively correlated with regional CBF (rCBF) in the left amygdala, lentiform nucleus, and parahippocampal gyrus, and positively correlated with rCBF in the right postero-lateral parietal cortex, whereas Milak et al. (2005) showed only positive correlations in bilateral mesiotemporal cortex, parts of the ventral subgenual basal forebrain, and most of the thalamus, hypothalamus, ventral striatum, and midbrain. Accordingly more studies are warranted to clarify the relationship between depression severity and brain activity including frontal lobe function.

In the present study, considering the consistent finding of attenuated oxy-Hb changes during VFT in the fronto-temporal regions in depression, we hypothesized that oxy-Hb changes during VFT in NIRS could be objective indicators of depressive symptom severity. Thus, we used multi-channel NIRS to investigate the relationship between oxy-Hb changes and symptom severity in patients with MDD. Because NIRS can be measured easily and noninvasively in a restraint-free environment over a short amount of time we expect that NIRS can be widely used to assess objectively depressive symptom severity as a clinical examination.

2. Materials and methods

2.1. Subjects

The subjects were 30 patients with MDD, and 30 healthy volunteers matched for age, gender and premorbid intelligence quotient (IQ). Premorbid IQ was estimated using the Japanese version of the National Adult Reading Test (Matsuoka et al., 2006). All subjects were right-handed according to the Edinburgh Inventory (Oldfield, 1971) and were native speakers of Japanese. All MDD subjects were outpatients of the National Center of Neurology and Psychiatry Hospital in Tokyo, Japan. They were diagnosed according to the Structured Clinical Interview for the Diagnostic Statistical Manual of Mental Disorders, 4th edition (DSM-IV) Axis I Disorders (SCID-I; First et al., 1995) by experienced psychiatrists. All patients were medicated with antidepressants. Twenty-seven out of 30 patients were prescribed with one or two antidepressants, 16 with SSRIs, 12 with tricyclics, 7 with milnacipran, 5 with tetracyclics, 2 with trazodone and 1 with mirtazapine. In addition, 20 patients were prescribed with anxiolytics, 16 with hypnotics, 7 with mood stabilizers and 9 with antipsychotics (Supplementary Table 1). Daily doses of all antidepressants were converted to an equivalent dose of imipramine (Inagaki and Inada, 2006) and anxiolytics/hypnotics to that of diazepam (Inagaki and Inada, 2006) for each patient. The controls were healthy volunteers recruited from the same geographical area through advertisements in free local magazines and our website announcement. They were interviewed using the SCID-I for MDD or SCID-NP for healthy volunteers and an unstructured interview for family history, and those individuals who had a current or past history of Axis I psychiatric disorder or a positive family history of Axis I psychiatric disorder within their first degree relatives were excluded. The exclusion criteria for both groups were previous head trauma, neurological illness, a history of electroconvulsive therapy, alcohol/substance abuse or addiction.

After the study procedures had been fully explained, written informed consent was obtained from every participant. This study was approved by the ethics committee of the National Center of Neurology and Psychiatry.

2.2. Clinical assessment

Depressive symptoms and the level of social functioning were evaluated by a single experienced psychiatrist using the GRID Hamilton Rating Scale for Depression 21-item version (GRID HAM-D21; Kalali et al., 2002) and Global Assessment of Functioning scores (GAF; American Psychiatric Association, 1994), respectively, without knowledge of the NIRS data on the same day that the NIRS measurements were conducted. Sleepiness was evaluated as the score on the Stanford Sleepiness Scale (SSS; Hoddes et al., 1973).

2.3. Activation task

The activation task was a letter version of VFT similar to that described by Takizawa et al. (2008). During the VFT, changes in oxy-Hb and deoxy-Hb were measured. The VFT consisted of a 30-sec pre-task baseline, a 60-sec VFT, and a 70-sec post-task baseline. The subjects were instructed to repeat the syllables /a/, /i/, /u/, /e/ and /o/ during the pre-task and post-task baseline periods. For the VFT, the subjects were instructed to generate as many words as possible.

One of the three initial syllables (A; 0–20 s /a/, /to/, or /na/, B; 20–40 s /i/, /ki/, or /se/, C; 40–60 s /o/, /ta/, or /ha/) was randomly

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presented on the computer display placed in front of the subjects, every 20 s during the 60-sec task. The number of possible combinations of syllables is 27 (A;3 × B;3 × C;3 = 27). We adopted 15 among the possible combinations. The number of correct words generated during the task was determined as a measure of task performance.

3. NIRS measurements

3.1. NIRS device

We used a 52-channels NIRS (ETG-4000 Optical Topography System; Hitachi Medical Co., Tokyo, Japan) which measures relative changes in oxy-Hb and deoxy-Hb using two wavelengths (695 nm and 830 nm) of infrared light based on the modified Beer–Lambert law (Yamashita et al., 1996). With this system, these Hb values include a differential pathlength factor (DPF). In the NIRS system, "hemoglobin concentration change*DPF" is calculated as a solution to the simultaneous equations based on the Beer–Lambert law, which cannot escape the effect of DPF. Although DPF varies among various brain regions Zhao et al., using a Monte Carlo simulation, reported the estimated DPF variation in the forehead region of adult humans was roughly homogeneous (Zhao et al., 2002).

The distance between a pair of source-detector probes was set at 3.0 cm and each area measured between a pair of source-detector probes was defined as a 'channel'. The NIRS device is considered to measure 'channels' at a 2–3 cm depth from the scalp, that is, at the surface of the cerebral cortex (Hock et al., 1997; Okada and Delpy, 2003, Toronov et al., 2001).

3.2. Probe positioning and measurement points

The NIRS probes were fixed with 3 × 11 thermoplastic shells, with the lowest probes positioned along the Fp1–Fp2 line according to the international 10–20 system used in electroencephalography. The probes can measure Hb values from bilateral prefrontal and temporal surface regions. The measuring points were labeled ch1 to ch52 from right-posterior to left-anterior (Fig. 1). The correspondence between these NIRS channels and the measurement points on the cerebral cortex was confirmed by a multi-subject study of anatomical cranio-cerebral correlations (Okamoto et al., 2004) and presented on the basis of results obtained by the virtual registration method (Tsuzuki et al., 2007).

3.3. Measurement parameters

The rate of data sampling was .1 second (s). The obtained data were analyzed using integral mode; the pre-task baseline was determined as the mean over a 10 s period just prior to the task period, and the post-task baseline was determined as the mean over the last 5 s of the post-task period. Linear fitting was then applied to the data between these two baselines. The moving average method using a window width of 5 s was applied to remove any short-term motion artifacts. Because we could not remove all artifacts in this way, we applied automatic rejection of data with artifacts separately for each channel (Takizawa et al., 2008).

According to the aforementioned measurement parameters for integral mode, the waveforms of oxy-Hb, deoxy-Hb and total-Hb

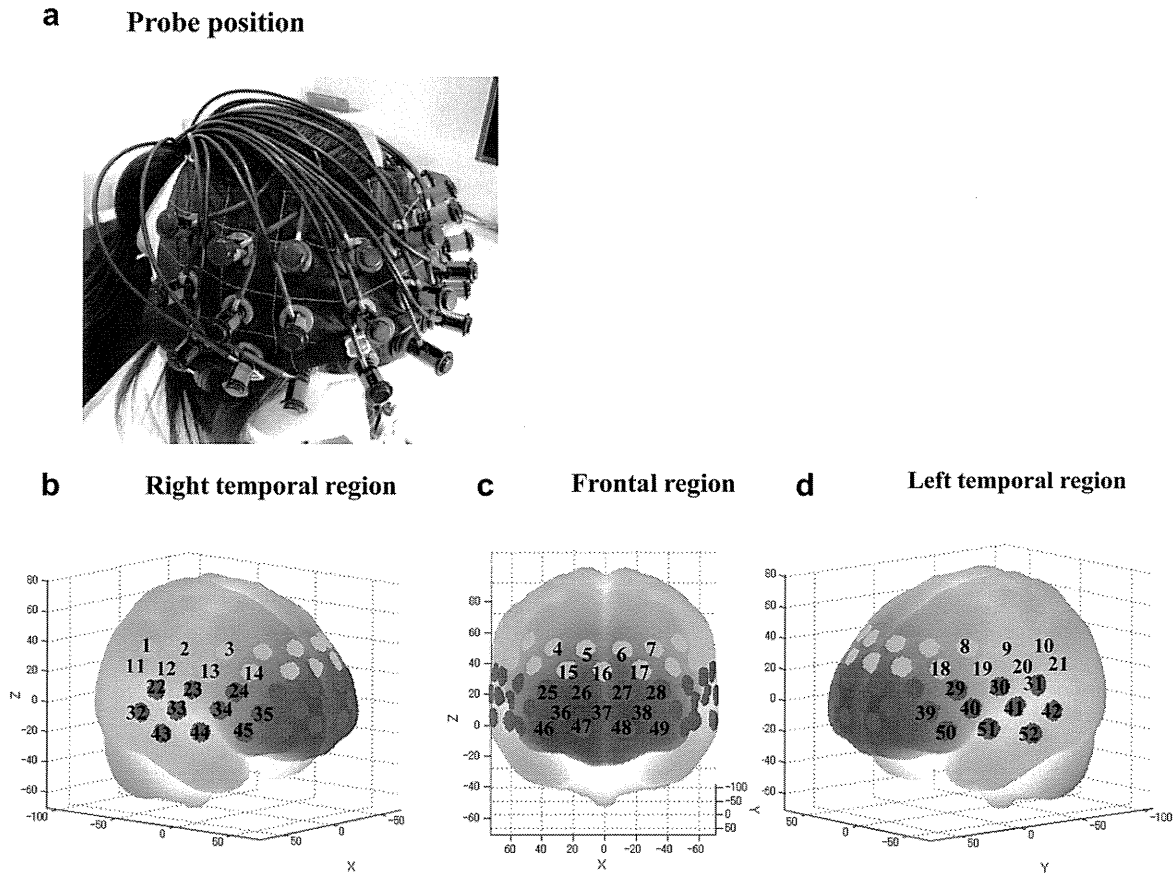

Fig. 1. Measurement points of 52 channels for near-infrared spectroscopy (NIRS) (a) Probes with 3 × 11 thermoplastic shells were placed over a subject's bilateral frontal regions. (b–d) The 52 measuring positions of the NIRS device are superimposed on the 3D-reconstructed cerebral surface, based on magnetic resonance imaging. The 52 measuring positions are labeled ch1 to ch52, from the right posterior to the left posterior. The dimensional figures b, c and d indicate the right temporal, frontal and left temporal brain regions, respectively. Because acquired NIRS data from the 21 channels in the upper two rows (pink channels) clearly contained artifacts presumably due to hair, as indicated by visual inspection of the waveforms, and signal to noise ratio seemed to be low, they were excluded from statistical analyses.

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changes were acquired from each subject in all 52 channels during VFT.

3.4. Measurement environment

The subjects sat on a comfortable chair in a silent and day-lit room. They were instructed to minimize motions such as head movements, strong biting and blinking during the NIRS measurement, to avoid artifacts.

Data clearly containing motion artifacts, based on both our observations and the NIRS recording, were excluded from further analyses.

4. Statistical analysis

Because acquired NIRS data from the 21 channels in the upper two rows clearly contained artifacts presumably due to hair, as indicated by visual inspection of the waveforms, and signal to noise ration seemed to be low, they were excluded from statistical analyses.

The χ^2 test or Student's *t*-test was used to compare proportions and means, respectively, between the MDD and control groups.

As for the analysis of the NIRS data, we focused on oxy-Hb data, since oxy-Hb change (task period − pre- and post-task baseline period) is assumed to more directly reflect cognitive activation than deoxy-Hb change as shown by a stronger correlation with blood-oxygenation level-dependent signal measured by fMRI (Strangman et al., 2002b). The mean oxy-Hb changes were compared between the two groups (MDD and control) for each channel using Student's *t*-test. To examine the relationships between oxy-Hb changes and HAM-D21 total scores, HAM-D21 subscale scores, GAF, or other clinical variables, Spearman's rhos were calculated for MDD patients.

All statistical analyses were performed using SPSS for Windows, version 18.0.0 software (SPSS Japan, Tokyo, Japan). A value of $p < .05$ (two-tailed) was considered to be statistically significant. We set the value of q specifying the maximum false discovery rate (FDR) at .05, such that the false positive rate was no more than 5% on average in treating the oxy-Hb data obtained from multiple channels (Singh and Dan, 2006).

5. Results

5.1. Demographic and clinical data of patients and controls

Table 1 summarizes demographic characteristics of the patients and controls. The two groups did not differ significantly in age, gender, handedness, estimated premorbid IQ or SSS.

5.2. Task performance

The number of words generated did not differ significantly among the 15 combinations employed (15 combinations: $F[1, 45] = 1.1$, $p = .39$; three initial syllables: $F[2, 90] = 1.2$, $p = .31$) in either group. The number of generated words during VFT did not differ significantly (patients: 12.3 ± 3.9; controls 13.9 ± 4.3, $t = 1.5$, df = 58, $p = .13$) between the MDD and control groups.

5.3. Group comparison

As shown in Fig. 2, the MDD group had significantly smaller oxy-Hb increases than the control group in 22 channels (ch22–29, ch32–33, ch35–39 and ch44-50; FDR-corrected *p*: .000–.024) during VFT.

5.4. Relationship with symptom severity at the time of examination

As shown in Fig. 2, there were significant negative correlations between mean oxy-Hb changes during the task and HAM-D21 total scores in one channel (ch25: rho = −.56; FDR-corrected *p*: .001). Mean oxy-Hb changes during the task period showed significant negative correlations with three individual items of the HAM-D21 subscale scores (Fig. 3); insomnia early in 9 channels (ch23, ch25–27, ch36–37 and ch46–48: rho = −.63 to −.46; FDR corrected *p*: .000–.014), work and activity in 2 channels (ch44 and ch45: rho = −.61 to −.57; FDR corrected *p*: .001 to .003), and psychomotor retardation in 12 channels (ch22–24, ch32, ch35–36, ch41, ch43–ch45, ch47 and ch51: rho = −.70 to −.44; FDR corrected *p*: .000–.018). Mean oxy-Hb changes showed no significant correlations with the remaining HAM-D21 subscale scores (i.e., depressed mood, guilt, insomnia middle, insomnia late, psychomotor agitation, anxiety psychic, anxiety somatic, loss of appetite, somatic symptoms general, sexual interest, hypochondriasis, loss of weight, insight, diurnal variation, and obsessional symptoms;) (Fig. 4).

Furthermore, mean oxy-Hb changes showed no significant correlation with task performance during VFT or other clinical variables, such as age, duration of illness, and sleepiness (data not shown).

5.5. Relationships with medication

There were no significant correlations between the HAM-D21 total score and doses of antidepressants (rho = −.23, $p = .22$) or anxiolytics (rho = .25, $p = .18$). There were significant negative correlations between mean oxy-Hb changes during the task and doses of antidepressants in 6 channels (ch31, ch40–41, ch45, ch50–51: rho = −.57

Table 1
Demographic and clinical data of patients with major depressive disorder and controls.

Demographics	Patients with depression ($n = 30$)	Healthy controls ($n = 30$)	Group difference *p*-value
Age (years)	36.7 ± 11.6	35.1 ± 9.4	.871
Gender (female/male)	16/14	16/14	1.000
Edinburgh handedness inventory (%)	92.9 ± 9.7	92.0 ± 11.5	.753
Age at onset (years)	30.9 ± 10.8	–	–
Duration of illness (years)	5.8 ± 4.1	–	–
Duration of medication (years)	5.0 ± 3.6	–	–
GRID HAM-D21 total score	16.7 ± 4.8	–	–
Estimated premorbid IQ	105.7 ± 9.5	105.9 ± 8.3	.953
Sleepiness	3.3 ± 1.1	2.9 ± .9	.104
GAF	57.6 ± 9.3		
Medication			
Imipramine equivalent dose (mg/day)	141.9 ± 127.6	–	–
Diazepam equivalent dose (mg/day)	8.5 ± 11.6	–	–

The χ^2 test or *t*-test was used to compare these variables between patients and controls. GAF, Global Assessment of Functioning; GRID HAM-D21, GRID Hamilton Rating Scale for Depression 21 item; IQ, Intelligence Quotient.

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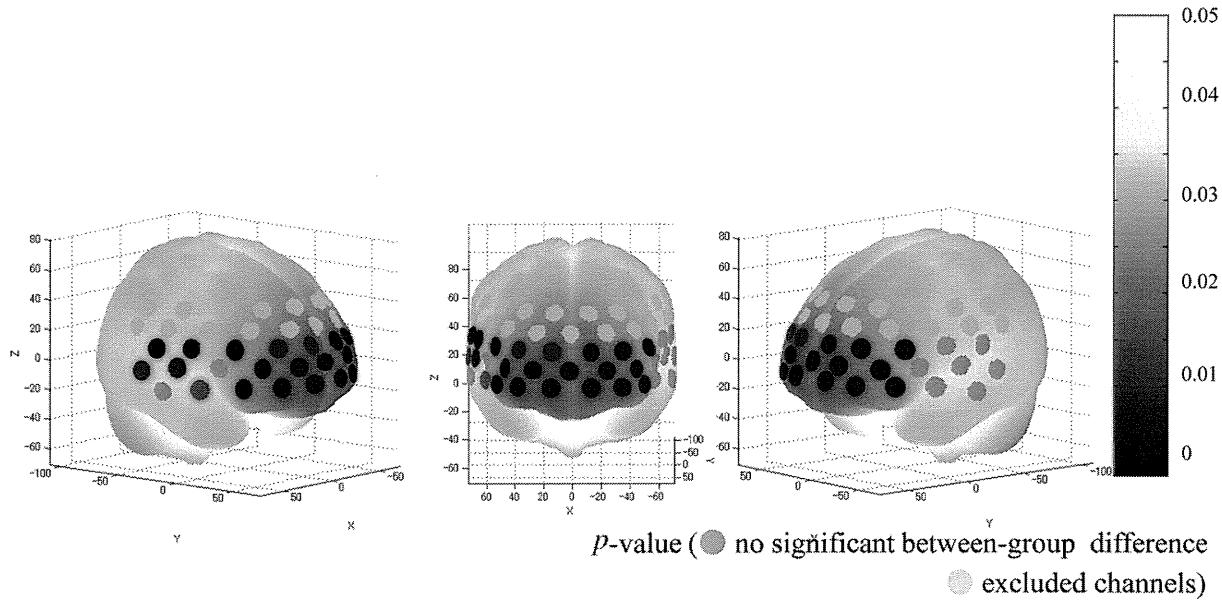

Fig. 2. *p*-value significance map of *t*-tests for oxy-Hb increases in patients with MDD compared with healthy controls during VFT using FDR correction. The warm colored circles represent significantly smaller oxy-Hb increases than in the control group at the channels indicated. There were 22 channels (ch22–29, ch32–33, ch35–39 and ch44–50; FDR-corrected *p*: 000–.024).

to −.48; FDR-corrected *p*: .002 to .007). Mean oxy-Hb changes showed no significant correlations with doses of anxiolytics.

6. Discussion

6.1. Task performance

The number of words generated during the VFT did not differ significantly between patients and controls, which is consistent with the majority of previous studies (Matsuo et al., 2002; Fossati et al., 2003; Suto et al., 2004; Kameyama et al., 2006). Previous studies reported impairment on semantic fluency tasks in depression (Calev et al., 1989; Tarbuck and Paykel, 1995). However, on phonemic fluency task conflicting results patients showing normal or impairment performance in depression (Albus et al., 1996; Degl'Innocenti et al., 1998). Type of psychiatric disorder and task time setting may reflect the discrepancies (Fossati et al., 2003). In the present study, the time setting of VFT was three phonemes within 60 s, that is, 20 s for each phoneme, which differs from the standard VFT usually using 60 s for one phoneme. The time setting condition was designed as it is, so that the subjects were able to keep generating words regularly within the task period to avoid the effect of "not speaking". It is possible that the time setting condition in the present study caused the lack of significant between group-difference in task performance.

6.2. Between-group comparison of oxy-Hb activation

The present study showed oxy-Hb activation during VFT to be significantly smaller in the MDD group than in age-, gender- and IQ-matched healthy controls. This result is essentially consistent with those obtained using NIRS (Matsuo et al., 2002; Herrmann et al., 2004; Suto et al., 2004; Kameyama et al., 2006; Pu et al., 2008), single photon emission computed tomography (SPECT) (Mayberg et al., 1994) or functional magnetic resonance imaging (fMRI) (Okada et al., 2003).

6.3. Relationships with symptom severity at the time of examination

Mean oxy-Hb changes during the task period showed a significantly negative correlation with HAM-D21 total score at ch25. Ch25 is located approximately in the right DLPFC. The finding is in line with some initial studies (Bench et al., 1995; Mayberg et al., 1994) which suggest that abnormal functions in DLPFC are mood dependent. However, other more recent studies investigating cross-sectional relationship between depression psychopathology and brain function do not coincide with our result (Périco et al., 2005; Milak et al., 2005). One of the reasons for the discrepancy may arise from the different methodologies; in the present study we adopted VFT for activation whereas the previous studies observed the basal activity with no activation task. Although speculative as it is, the activation of PFC by VFT may have led to the significant relationship between oxy-Hb changes and depression symptom severity in the right DLPFC.

More interestingly, mean oxy-Hb changes during the task period showed significant negative correlations with three individual HAM-D21 items in a wider area than they showed with HAM-D21 total scores; insomnia early in nine, work and activity in two and psychomotor retardation in twelve channels. The nine channels correlating with "insomnia early" were located approximately in the right pre-motor area, DLPFC and frontopolar and orbitofrontal areas. The two channels correlating with "work and activity" were located approximately in the right DLPFC and temporopolar area. The twelve channels correlating with "psychomotor retardation" were located broadly in the fronto-temporal areas with right hemispheric dominance. Although these findings should be treated with care given the exploratory nature of multiple analyses, it is noteworthy that at least some subscale scores of HAM-D21 appeared to show stronger relationship with oxy-Hb changes than HAM-D21 total scores. It has been pointed out that HAM-D17 and/or HAM-D21 are not necessarily unidimensional, and thus not adequate to assess depression severity (Bagby et al., 2004). Licht et al. (2005) showed that a set of the HAM-D containing six subscales constitute a unidimensional scale measuring severity of

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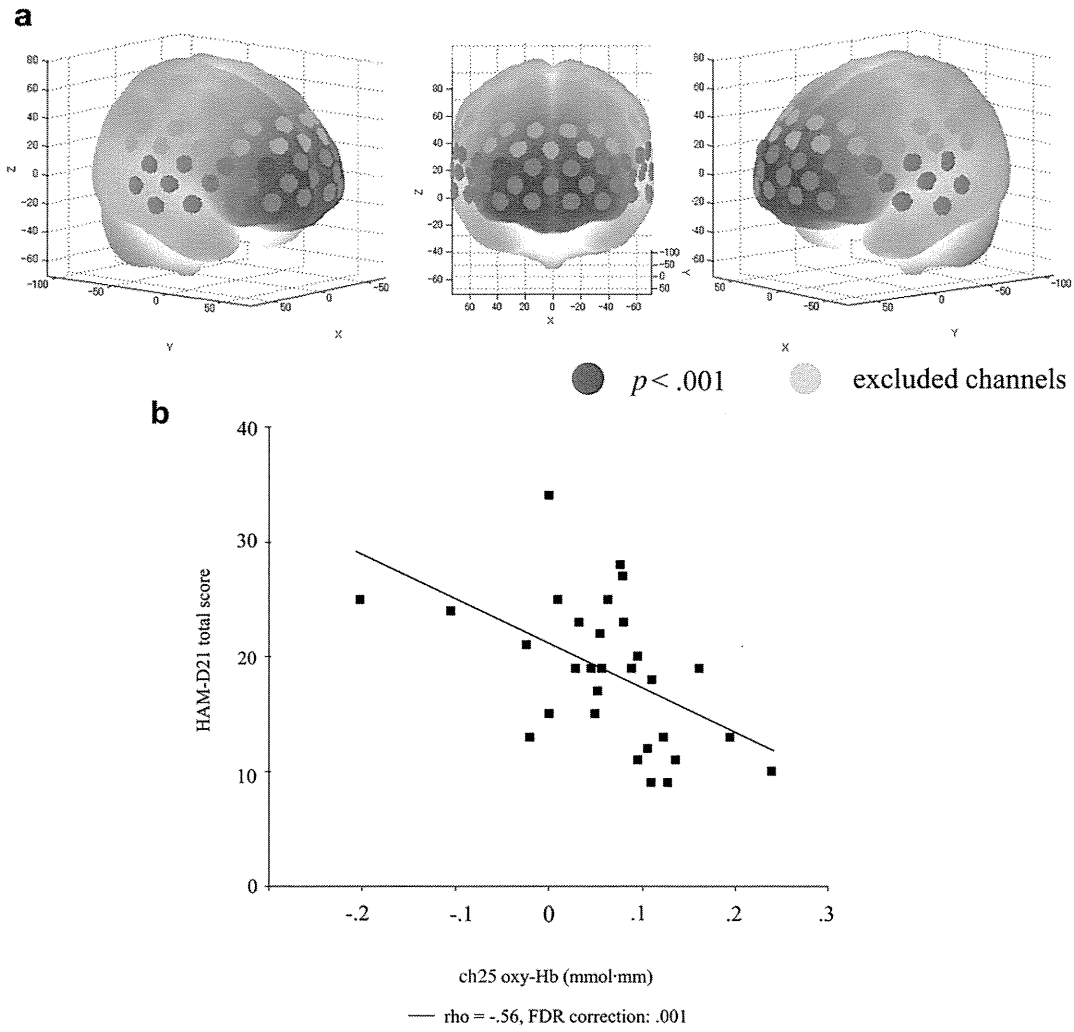

Fig. 3. (a) The channels with a significant correlation between oxy-Hb changes and HAM-D21 total score after FDR correction. (b) Scatter graph showing the relationship between HAM-D21 total scores and oxy-Hb activation in ch25.

depression, whereas the remaining items covering neurovegetative symptoms showed a problematic response somewhat insensitive to depression severity. In fact, the multidimensionality was highlighted in the unstable factor structure, which was demonstrated by a failure to replicate a single unifying structure across studies (Bagby et al., 2004). The relatively strong relationship indicated between HAM-D21 subscale scores and oxy-Hb changes in divergent areas, compared to HAM-D21 total scores may be due to the multidimensional properties of HAM-D21. Graff-Guerrero et al. (2004) also demonstrated that each HAM-D subscale score showed a significant correlation with the basal CBF in variant areas, in some cases showing positive correlation and others negative.

6.4. Relationships with medications

As all patients were taking antidepressants at the time of evaluation, the medication effect could not be ignored. Yet, there was no significant relationship between daily dose levels of antidepressants and the HAM-D21 total score. Although daily dose levels of antidepressants showed significant negative correlations with oxy-Hb changes in six channels, ch25, where a significant correlation between oxy-Hb changes and HAM-D21 total scores was observed, was not included in the six channels. Therefore, we suspect that the effect was small, if at all.

PET has been used to demonstrate that antidepressant medication normalizes both over-activity and under-activity in the frontal cortex (Kennedy et al., 2001, 2007; Mayberg et al., 2000; Goldapple et al., 2004). Unfortunately, our results could not clarify the relationship between medication and brain activation because our analysis was based on cross-sectional data. Although our data may reflect the more restraint-free, natural setting than those using fMRI or PET, further studies in drug-naïve patients are required to draw any conclusions as to the possible effects of medication on brain activation as measured by NIRS. Longitudinal studies investigating the relationship between the change in oxy-Hb data and symptom severity scores with a larger sample size are warranted to reach a conclusion on this matter.

The results of this study must be interpreted with caution due to certain limitations. First, because the analysis was based on cross-sectional data, causality cannot be determined. Longitudinal studies are needed to assess cause-and-effect relationships. Second, our sample size was not large, and is thus subject to type II error. Further studies with larger numbers of MDD patients are required. Finally, owing to the multidimensional properties of HAM-D21, assessment of depression symptom severity using HAM-D21 total scores may not be adequate, and thus, other scales such as Montgomery Asberg Depression Rating Scale (MADRS) or Beck Depression Inventory (BDI) should be tested in the future study.

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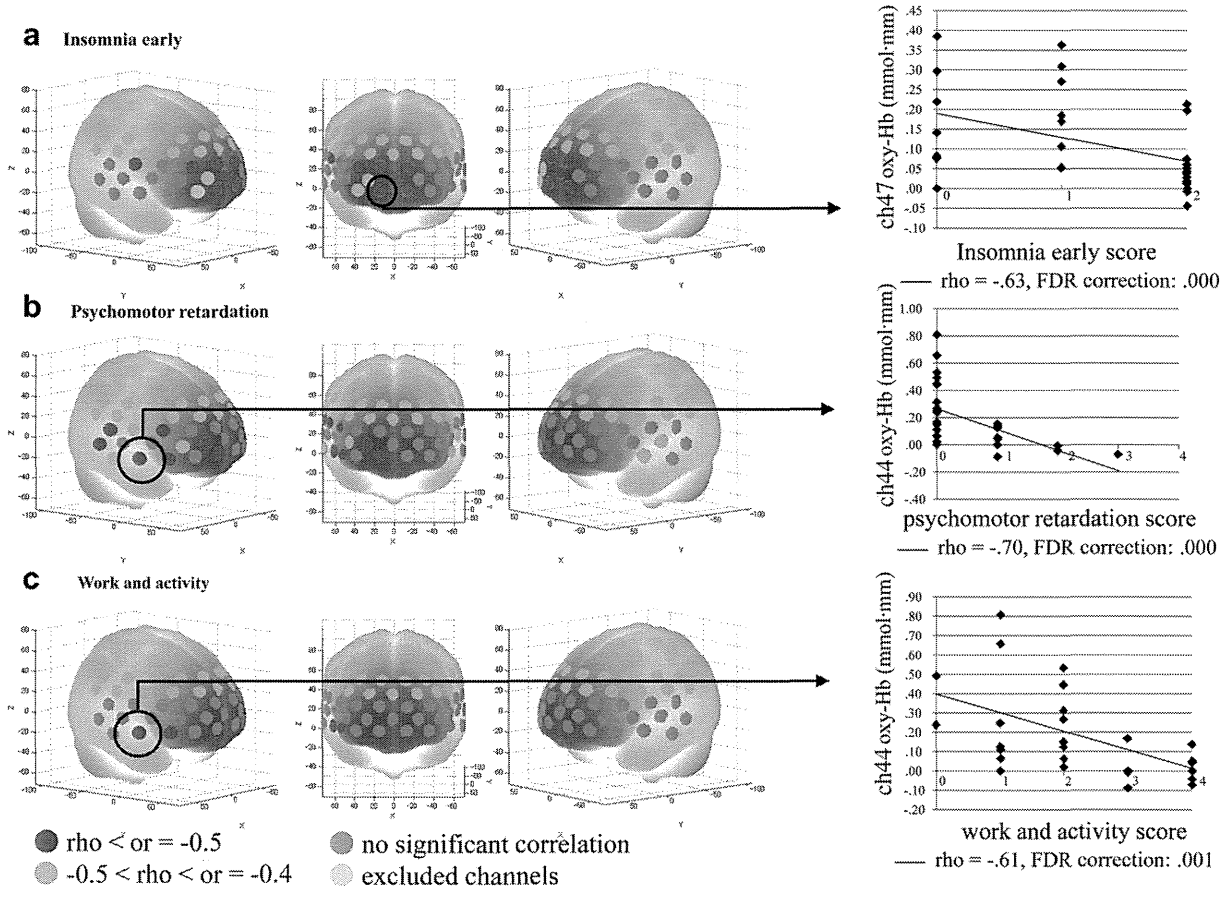

Fig. 4. rho-value map for the correlation between oxy-Hb activation in MDD patients and three individual HAM-D21 subscale scores after FDR correction. (a) insomnia early, (b) psychomotor retardation, and (c) work and activity.

7. Conclusion

In this study, we confirmed that the increase in oxy-Hb during a VFT task is significantly smaller in MDD than in age- and gender-matched healthy subjects. This difference could not be explained by a difference in task performance or premorbid IQ. The blunted increase in right DLPFC was associated with the symptom severity of MDD and therefore oxy-Hb changes during VFT in this region may be a state-dependent marker of depression.

Role of the funding source

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Contributors

T. Noda designed the study, wrote the protocol, assessment of depression severity, literature searches, statistically analyzed the data, and wrote the first draft of the manuscript. T. Matsuda was involved in patient recruitment and assessment of depression severity. H. Kunugi and S. Yoshida wrote the final version of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All the authors declare that they have no conflicts of interest with respect to this study or its publication.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpsychires.2012.04.001.

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精神疾患の診断ツールとしての光トポグラフィー

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Key words：近赤外線光トポグラフィー（near-infrared spectroscopy：NIRS）、鑑別診断、先進医療、大うつ病性障害、双極性障害、統合失調症

【要旨】　精神疾患の患者数は増え続け、現在323万人と4大疾病それぞれの患者数よりも多く、2011年に精神疾患は、医療計画に記載すべき疾患に追加され5大疾病となった。増え続ける精神疾患であるが、診断を問診に頼らざるを得ないことから、客観的な診断が得られない問題点も指摘され、客観的な診断・評価ツールの開発が望まれていた。近赤外線光トポグラフィー（near-infrared spectroscopy：NIRS）は近赤外光を用いており、非侵襲性、低拘束性、簡便に検査ができるという他の脳機能画像検査よりも優れた特徴を持つ一方で、光路長の問題や深部構造までは計測ができないという短所も併せ持つ。簡便に検査ができ、低拘束性、非侵襲性というストレスが少ない点は、精神疾患患者の臨床検査として大きな長所と考えられる。さらに、精神疾患を対象とした研究結果から、診断補助としての有用性が評価され、2009年4月に先進医療「光トポグラフィー検査を用いたうつ症状の鑑別診断補助」として承認され、2012年3月現在14施設で実施している。実際の臨床においては、診断補助ツールとしての有用性だけでなく、患者と共有できる客観的な情報がもたらされることは大きなメリットである。とくに患者との治療関係において、こうした情報が共有されることによって、病識の獲得や、病状の理解につながり、患者の積極的な治療参加がもたらされることが期待される。

はじめに

2008年の患者調査によると、精神疾患の患者数は323万人であり、4大疾病の患者数（悪性新生物152万人、脳血管疾患134万人、虚血性心疾患81万人、糖尿病237万人）よりも多い。また、2009年の人口動態統計によると、精神疾患による死亡数は1.1万人、自殺による死亡者数3.1万人となっているが、自殺者の約9割が精神疾患に罹患していたとの報告[1]もあり、精神疾患による死亡者数は統計データよりも多いことが推測される。このような背景から2011年に精神疾患が医療計画に記載すべき疾患に追加され5大疾病となった。急増する精神疾患であるが、問診によって診断を行うため客観性が乏しいことが指摘されてきた。患者との治療関係という側面では、1）患者が病識を持ちにくい疾患であること、2）診断の客観性が乏しいこと、から患者と病状の理解を共有することが難しく、治療へのスムーズな移行ができない場面もあった。そのため、精神科の臨床場面では客観的な診断ツールの臨床応用が長年にわたり期待されてきた。

近赤外線光トポグラフィー（near-infrared spectroscopy：NIRS）は機能的核磁気共鳴画像法（functional magnetic resonance imaging：fMRI）やポジトロン断層法（positron emission tomography：PET）、シングルフォトン断層法（single photon emission tomography：SPECT）などと同様に脳機

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能画像検査のひとつであり、1990年代から臨床研究に応用されてきた。NIRSは計測に近赤外光を使用しているため非侵襲的で、被験者の拘束が少ないため乳幼児からも測定可能である。また、連続した測定や繰り返し測定にも対応できる。被験者の拘束が少ないという点は日常生活に近い自然な状態の脳機能計測ができるという長所があり、精神症状から日常生活場面で支障をきたす精神疾患患者の脳機能測定には適当な検査であると考えられる。

精神疾患を対象としたNIRS研究はOkadaらが1994年に統合失調症を対象とした報告[2]に始まり、その後、Sutoら[3]、Kameyamaら[4]、Takizawaら[5]が言語流暢性課題を施行した際の前頭部における酸素化ヘモグロビン（oxy-Hb）の変化が精神疾患毎に独特のパターンを示すという報告がされ、先進医療の承認につながった。本稿では精神疾患の診断ツールとして、先進医療に承認されたNIRSの鑑別診断補助における有用性および課題について紹介する。

I. NIRSの特徴

1）近赤外光

近赤外光は生体を透過しやすく、ヘモグロビンは近赤外光を吸収しやすいという特徴がある。oxy-Hbと脱酸素化ヘモグロビン（deoxy-Hb）はそれぞれ近赤外光の吸収係数が異なるため、二つの波長の近赤外光を用いることで両者の濃度変化を推定できる。脳機能測定をするには近赤外光を頭皮から照射することになる。近赤外光は頭皮、筋肉、頭蓋骨、硬膜、軟膜、髄液、脳実質などの影響を受け、吸収されたり散乱するため、光路長を正確に測定できない。

2）脳機能計測

脳機能の計測は大きく分けると、脳波などのように脳内で発生した電気信号を直接計測する方法と、NIRSやfMRI、PET、SPECTなどのように神経活動に伴う脳血流、血液量の変化を計測する方法の二つの方法がある。後者は神経血管カップリング（neurovascular coupling）理論に基づいている。NIRSでは毛細血管や静脈側のヘモグロビンを計測しており[6]、動物実験によると脳血流変化とoxy-Hb濃度の変化とが高い相関を示している[7]ため、NIRSにおいてはoxy-Hb濃度が脳活動を反映すると考えられている。

3）NIRSの利点と限界

NIRSは近赤外光を用いているため様々な長所がある。①近赤外光は生体を透過しやすく、非侵襲的であるため安全性が高い、②MRI計測などに伴う拘束が必要でないため、より自然な姿勢で検査ができる。その結果、少ないストレスで計測が可能となる、③操作が簡便である、④静かな環境での計測が可能である、⑤サンプリングが0.1秒毎と比較的高い時間分解能をもつ。とくに自然な姿勢で検査できることは、患者への心理的負担を減らすことができ、精神疾患患者を対象とする場合に大きな利点であると言える。一方、NIRSの限界は光路長を正確に計測できない点[8]にある。そのため、ヘモグロビン濃度の絶対値を計測することはできない。また、測定可能な脳領域は頭皮から2cm程度の深さまでであり、MRIやPETと比較して空間分解能は低い[8]。

II. 先進医療

これまで精神科の臨床場面では、血液検査や心電図検査のような診断の参考になるような客観的な検査がなく、問診によって得られた情報から診断を行っていた。診断や病状に基づいて治療方法が決定されるため、診断の重要性は明白である。診断の参考となる客観的な検査法がなかったことから、患者と医療者とで病状や診断の理解が共有できにくく、患者の治療への積極的な参加の妨げとなることが問題であった。可視化できる検査情報を患者と共有でき、病状や治療必要性の理解が向上し、治療意欲の強化につながることが期待される。NIRSを用いた遂行機能課題中に健常者、

大うつ病性障害、双極性障害、統合失調症毎に異なった脳血液量変化を示すという研究結果[3-5,9-11]、うつ症状を呈する大うつ病性障害、双極性障害、統合失調症を6~8割の精度で鑑別が可能であるという心の健康に光トポグラフィー検査を応用する会による多施設共同研究の結果[11,12]から診断補助ツールとしての有用性が評価され、2009年4月に先進医療「光トポグラフィー検査を用いたうつ症状の鑑別診断補助」として承認された。先進医療の対象疾患は「国際疾病分類第10版（ICD-10）においてF2（統合失調症・統合失調型障害および妄想性障害）に分類される疾病およびF3（気分（感情）障害）に分類される疾病のいずれかの疾病の患者であることが強く疑われるうつ症状の者（器質的疾患に起因するうつ症状の者を除く）に係るものに限る」である。先進医療の実施施設は2012年3月時点で14施設、2010年7月から2011年6月の1年間の実施件数は703件であった。なお、国立精神・神経医療研究センター病院では2009年10月に光トポグラフィー（NIRS）専門外来を開設し外来を中心に先進医療を提供している。

III. NIRSの測定

国立精神・神経医療研究センター病院では、先進医療「光トポグラフィー検査を用いたうつ症状の鑑別診断補助」の測定に52チャンネルNIRS装置（ETG-4000, 日立メディコ社製）を用いている（図1）。測定範囲は前頭部から左右側頭部である。測定課題はSutoら[3]、Kameyamaら[4]が報告した言語流暢性課題（verbal fluency task：VFT）を用いている。通常用いられる測定条件である60秒間のVFTでは、被験者が発語しなくなることによるNIRS信号への影響を小さくするため、NIRS測定時のVFTでは、60秒間を3分割し、20秒ごとに3つの異なる頭文字を提示する。なお、VFT前後は統制課題としてVFT前に30秒間、VFT後に70秒間「あ・い・う・え・お」を繰り返すという課題を使用している。計測は課題に集中するために専用の防音室で行っている。体動などのアーチファクトが混入しやすいため、事前に被験者へ説明して協力を依頼するとともに練習することでsignal/noise比（S/N比）を高めるよう努力している。実際にNIRS測定で得られた波形を判読する際に、アーチファクトの混入によって判読ミスが生じることがある。先進医療を行うにあたり、測定時にS/N比を高めること、アーチファクトを見極めることが判読ミスを減らす上で求められる。

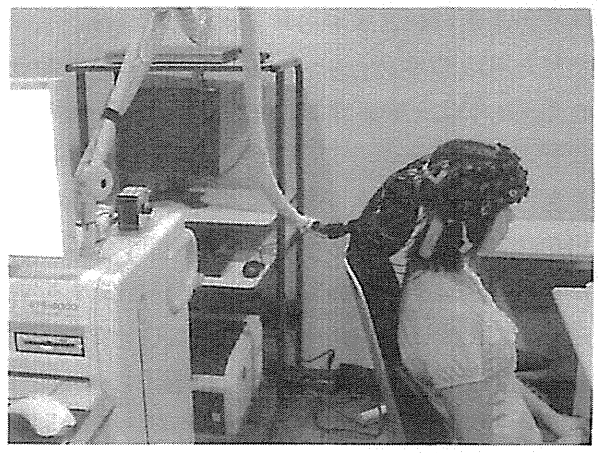

図1. NIRS測定風景

IV. 大うつ病性障害、双極性障害、統合失調症のNIRS研究

大うつ病性障害および双極性障害を対象とした研究を概観すると、結果は概ね一致していると言える。1996年にOkadaらが健常被験者と大うつ病性障害患者を対象とした鏡映描写課題（Mirror Drawing Task：MDT）を用いた報告が最初である。課題中大うつ病性障害患者の半分は劣位半球で優位な反応が認められ、健常被験者では優位半球が強く反応したという結果とは異なっていた[2]。2002年にMatsuoらが寛解状態の大うつ病性障害と双極性障害を対象として、健常被験者をコントロール群としたNIRSを用いた研究を報告した。