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資　料

Multichannel Near-Infrared Spectroscopy in Depression and Schizophrenia: Cognitive Brain Activation Study

Tomohiro Suto, Masato Fukuda, Makoto Ito, Toru Uehara, and Masahiko Mikuni

Background: *Recent developments in near-infrared spectroscopy (NIRS) have enabled the noninvasive clarification of brain functions in psychiatric disorders with measurement of hemoglobin concentrations as cerebral blood volume.*

Methods: *Ten patients with depression, 13 patients with schizophrenia, and 16 age- and gender-matched healthy control subjects participated in the study after giving consent. The relative concentrations of oxyhemoglobin [oxyHb] were measured with frontal and temporal probes every .1 sec during word fluency and unilateral finger tapping tasks, with two 24-channel NIRS machines.*

Results: *The [oxyHb] increase patterns during the word fluency task varied among the three groups, although their task performances were similar: the depression group was characterized by a smaller [oxyHb] increase during the first half of the task period and the schizophrenic group by a small trough of [oxyHb] at the start of the task period and [oxyHb] re-increase in the posttask period. [OxyHb] increases during the finger-tapping task were rather larger in the patient groups than in the control group.*

Conclusions: *The characteristic time courses of [oxyHb] changes in the frontal lobe were elucidated for depression and schizophrenia. Near-infrared spectroscopy, with its noninvasiveness and high time resolution, can be a useful tool for research and clinical purposes in psychiatry.*

Key Words: Near-infrared spectroscopy, cerebral blood volume, depression, schizophrenia, word fluency test, diagnosis

Neuroimaging studies have revealed structural and functional brain abnormalities in psychiatric disorders. Schizophrenia was reported to be characterized by volume reductions of several structures in frontal and temporal lobes, as reviewed by Shenton et al (2001). Schizophrenia was also found to show two types of abnormal brain function (Weinberger et al 2001) in many functional neuroimaging studies using positron emission tomography (PET), single-photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI): "hypofrontality" (i.e., decreased activation in glucose metabolism and regional cerebral blood flow [rCBF] in the frontal lobes in most of the so-called frontal tasks (reviewed by Andreasen et al 1998), and "hyperfrontality" (i.e., increased activation in glucose metabolism and rCBF) if task performances in schizophrenic patients were matched with those in healthy controls (summarized by Weinberger et al 2001). Mood disorders are also characterized by structural and functional brain abnormalities. They include volumetric reduction in the subgenual prefrontal cortex (Drevets et al 1997), decreased rCBF and glucose metabolism in the dorsolateral prefrontal cortex, and increased rCBF and glucose metabolism in the orbital and medial prefrontal cortices, compared with those observed in healthy subjects (Brody et al 2001; Drevets 2000; Liotti and Mayberg 2001).

Functional brain imaging methodologies, such as PET, SPECT, and fMRI have the disadvantage of requiring large apparatuses. This prevents their use in a bedside setting for diagnostic and treatment purposes. Recently, the development of near-infrared spectroscopy (NIRS) has enabled noninvasive and bedside measurements of regional cerebral blood volume (rCBV) in terms of the relative concentrations of oxyhemoglobin [oxyHb] and deoxyhemoglobin [deoxyHb], with a high time resolution. Near-infrared spectroscopy is based on the principle that near-infrared light is preferentially absorbed by [oxyHb] and [deoxyHb] and not so much by other body tissues. Near-infrared light emitted from the skin travels into the body, is reflected and absorbed by body tissues, and reappears on the skin. The absorption of near-infrared light thus reflects Hb concentrations in the tissue beneath emission and detection probe pairs. Measurements with two or more wavelengths of near-infrared light enable the determination of [oxyHb] and [deoxyHb] changes, because their absorptions vary along its wavelength. The successful monitoring of brain functions in humans with NIRS was first reported in four studies (Chance et al 1993; Hoshi and Tamura 1993; Kato et al 1993; Villringer et al 1993), and multichannel NIRS machines were developed in the late 1990s (Maki et al 1995; Tamura et al 1997).

The [oxyHb] increase and [deoxyHb] decrease in NIRS have been shown to reflect cortical activation by simultaneous measurements with other methodologies. For example, high correlations were obtained between [oxyHb] increase and rCBF change in a $^{15}H_2O$ PET study (Hock et al 1997) and between [deoxyHb] decrease (Kleinschmidt et al 1996, Mehagnoul-Schipper et al 2002, Toronov et al 2001) or [oxyHb] changes (Strangman et al 2002b) and cerebral blood oxygenation increase in MRI studies.

Near-infrared spectroscopy has advantages and disadvantages over other methodologies. The advantages of NIRS are 1) near-infrared light is completely noninvasive, hence repeated measurements are possible; 2) Hb data obtained have a time resolution on the order of .1 sec, which is superior to those of other imaging methodologies for cerebral blood flow and metabolism but is inferior to those of event-related potentials and magnetoencephalography, which directly measure electrical activity of neurons with a time resolution on the order of milliseconds; 3) subjects are under natural conditions during examination so that they can perform the task; and 4) apparatuses are small and portable. The disadvantages of NIRS are that it enables

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Table 1. Characteristics of the Subjects

Major Depressive Disorder

Case No.	Age	Sex	I/O	Course	PS	MS	HAMD	Medication (mg/day)	WFT
DP1	24	M	I	SE	Absence	Presence	6	Mianserin 20, paroxetine 40, carbamazepine 400	14
DP2	30	M	O	RE	Absence	Presence	2	Amitriptyline 100	15
DP3	51	M	O	SE	Absence	Presence	2	Amitriptyline 25, lithium 800, bromocriptine 7.5	16
DP4	52	M	O	RE	Presence	Absence	0	Clomipramine 10, trazodone 25	23
DP5	54	M	O	SE	Absence	Absence	12	Trazodone 50, paroxetine 40	9
DP6	55	M	I	RE	Presence	Presence	8	Maprotiline 100, milnacipran 150	22
DP7	57	M	O	SE	Absence	Absence	1	Mianserin 20	5
DP8	59	M	O	SE	Absence	Presence	17	Milnacipran 45	17
DP9	60	M	O	RE	Absence	Presence	19	Mianserin 30, fluvoxamine 150, levomepromazine 10, carbamazepine 200, L-thyroxine .05	16
DP10	37	F	O	RE	Absence	Presence	18	Clomipramine 125, levomepromazine 10	9
Mean	47.9	M9/F1	I2/O8	SE5/RE5	Presence 2/ Absence 8	Presence 7/ Absence 3	7.5		14.6
SD	12.8						6.7		5.7

Schizophrenia

					PANSS[b]				
Case No.	Age	Sex	I/O	Subtype	+	−	+/−	Medication (mg/day)	WFT
SC1	23	M	O	P	10	24	34	Risperidone 2	16
SC2	26	M	O	R	9	10	2	Chlorpromazine 50, haloperidol 3	19
SC3	28	M	O	R	15	14	29	Levomepromazine 10, risperidone 6	19
SC4	28	M	O	P	11	10	28	Chlorpromazine 100, nemonapride 40, olanzapine 5	19
SC5	28	M	O	P	21	26	43	Risperidone 8	10
SC6	37	M	O	R	9	18	16	Chlorpromazine 50, bromperidol 27, olanzapine 10	17
SC7	40	M	O	P	15	15	23	Chlorpromazine 25, levomepromazine 50, risperidone 2	9
SC8	50	M	O	R	18	23	41	Chlorpromazine 150, bromperidol 6, risperidone 9	17
SC9	57	M	O	R	9	11	26	Chlorpromazine 175, haloperidol 3	16
SC10	29	F	O	R	9	14	22	Risperidone 2	16
SC11	42	F	O	P	12	12	22	Quetiapine 50	10
SC12	49	F	O	P	14	12	13	Haloperidol 4.5, zotepine 300	13
SC13	56	F	O	P	12	10	16	Levomepromazine 5, bromperidol 15, perospiron 32	15
Mean	37.9	M9/F4	I0/O13	P7/D0/C0/	12.6	14.5	26		15.1
SD	12			U0/R6	3.8	5.9	8.5		3.5

Control Subjects (n = 16)

	Age	Sex	WFT
Mean	42.9	M12/F4	16.8
SD	4.6		3.6

I/O, Inpatient/outpatient; SE, single episode; RE, recurrent episode; PS, psychotic symptoms; MS, melancholic symptoms; HAMD, Hamilton Rating Scale for Depression; WFT, word fluency task; M, male; F, female; P, paranoid; R, residual types; D, disorganized; C, catatonic; U, undifferentiated; PANSS, Positive and Negative Syndrome Scale; +, positive; −, negative; +/−, general.

measurement of Hb concentration changes 1) only as relative values, not as absolute values; 2) only in the cortex immediately beneath the probes but not in deeper brain structures; 3) with a high time resolution but with a poor spatial resolution; and 4) not only in the brain but also in more surface structures, such as the skin and skull.

Considering the advantages and disadvantages described above, NIRS is assumed to be particularly useful in assessing the dynamic aspects of cortical activation in rather broad areas. Based on these characteristics of NIRS, NIRS studies have been carried out for motor and cognitive activations in healthy and psychiatric subjects, as reviewed by Koizumi et al (1999), Obrig and Villringer (2003), and Strangman et al (2002a). We have found that the time courses of [oxyHb] changes during a motor task varied depending on the measuring channel and that interindividual differences in the amplitude of [oxyHb] increase determined with multichannel NIRS machines correlated with subject personality (Ito et al, unpublished data).

Most NIRS studies demonstrated activation-related [oxyHb] and [totalHb] (sum of [oxyHb] and [deoxyHb]) increases during language tasks (Hock et al 1997; Matsuo et al 2000, 2002; Sakai et al 2000; Sato et al 1999; Watanabe et al 1998; Yamamoto et al 1999) and cognitive tasks (Fallgatter and Strik 1997, 1998; Hock et al 1995; Hoshi et al 2000; Tamura et al 1997), as well as during motor tasks (Colier et al 1999; Hirth et al 1997; Maki et al 1995; Obrig et al 1996). Fallgatter et al (1998) reported, however, that

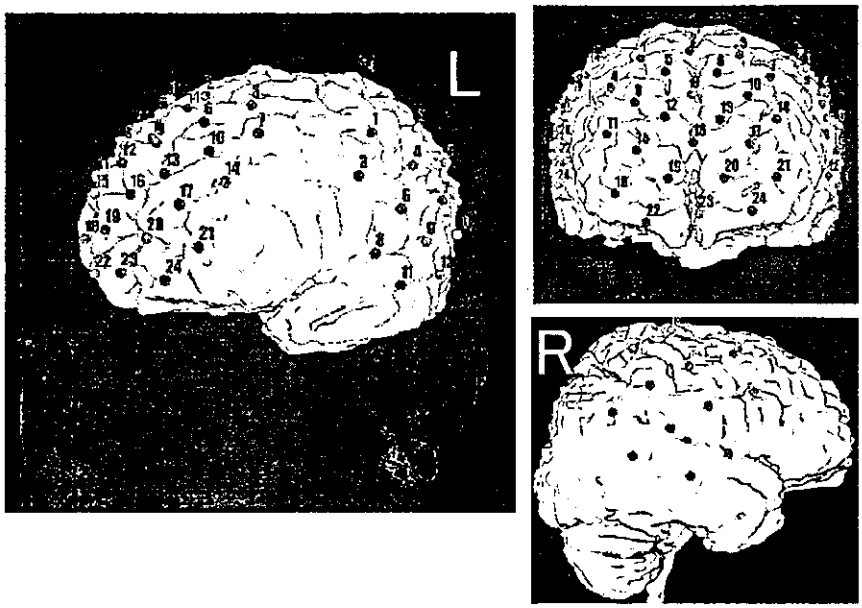

Figure 1. Cortical projection at near-infrared spectroscopy (NIRS) measurement positions. The measurement positions of the NIRS machines are superimposed on a magnetic resonance image of a reconstructed cerebral cortex of a representative subject.

[deoxyHb] increases and [oxyHb] decreases during a reading-aloud task. Thus, [deoxyHb] changes are less consistent, in that both increases and decreases in [deoxyHb] have been reported.

Near-infrared spectroscopy has only recently been applied to the examination of psychiatric patients as well as healthy subjects, and some NIRS studies of depression, schizophrenia, and other psychiatric disorders have recently been reported. Regarding depression, hemispheric asymmetry and reduced hemodynamic response during cognitive activation have been suggested. Okada et al (1996) found altered hemispheric differences in [totalHb] changes in the frontal region of depressive patients during a mirror drawing task. Matsuo et al (2000, 2002) found, with some inconsistencies, in patients with major depressive disorder and bipolar disorder that both the extent of [oxyHb] increase during a word fluency task and that of [oxyHb] decrease during hyperventilation tend to be reduced. Such reduced [oxyHb] increases in depression were reported to predict good therapeutic efficiency of repetitive transcranial magnetic stimulation (Eschweiler et al 2000).

As for schizophrenia, characteristic Hb changes were indicated in some NIRS studies. Okada et al (1994) found dysregulated hemispheric differences in [oxyHb] and [deoxyHb] during a mirror drawing task. Fallgatter and Strik (2000) also found that hemispheric asymmetry of [deoxyHb] during the Continuous Performance Test is reduced in schizophrenic patients as compared with healthy subjects. Other psychiatric and neurologic applications of NIRS include the study of Alzheimer's dementia (Hock et al 1997) and the determination of the language-dominant hemisphere before neurosurgical operation in epileptic patients (Watanabe et al 1998).

All the NIRS studies on psychiatric patients cited above suffer from the paucity of channels for measurements: the NIRS machine used is composed of one to a few channels. Considering the observation that Hb changes monitored from above the skull vary markedly depending on the measuring point, simultaneous measurements from multiple channels during activation are desirable for the assessment of specific findings in psychiatric populations.

In the present study, we used multichannel NIRS machines to examine the temporal and topographical characteristics of rCBV changes during cognitive activation in patients with major depressive disorder, patients with schizophrenia, and gender- and age-matched control subjects. Our aim was to assess brain dysfunctions in psychiatric disorders along the time course; that is, in addition to the frontal lobe dysfunction findings as revealed by PET, SPECT, and fMRI studies expressed as mean decreases in cerebral blood flow and metabolism rate across the task period employed, patients with depression and schizophrenia would show characteristic time courses of rCBV changes across the task period. We also hypothesized that such a brain dysfunction is task-demand-specific; thus, we used motor activation as a control task for cognitive activation.

Methods and Materials

Subjects

The subjects were 10 patients with major depressive disorders, 13 patients with schizophrenia, and 16 healthy control subjects. All patients were inpatients or outpatients of the Department of Neuropsychiatry, Gunma University Hospital in Gunma, Japan. They were diagnosed according to DSM-IV criteria (American Psychiatric Association 1994). Table 1 summarizes the demographic characteristics, psychopathology, courses of the illnesses, and medication dosages of the subjects.

All the patients were 23–60 years old, and were taking psychotropic drugs at the time of examination. Their psychopathology was assessed with the 24-Item Hamilton Rating Scale for Depression (Hamilton 1960) for major depressive disorders and the Positive and Negative Syndrome Scale (Kay et al 1998) for schizophrenia. The patients generally had mild symptomatology or were in remission, and at most they were taking moderate doses of psychotropic drugs.

The healthy control subjects included 12 men and four women (mean age 42.9 years, SD 4.6, range 36–52). They had no history of schizophrenia, mood disorders, epilepsy, or other psychiatric disorders. Based on the checklist filled out by these healthy subjects, we excluded those who were taking any medications or had a history of a major physical illness, neurologic disorder, substance abuse, alcohol abuse, or head trauma.

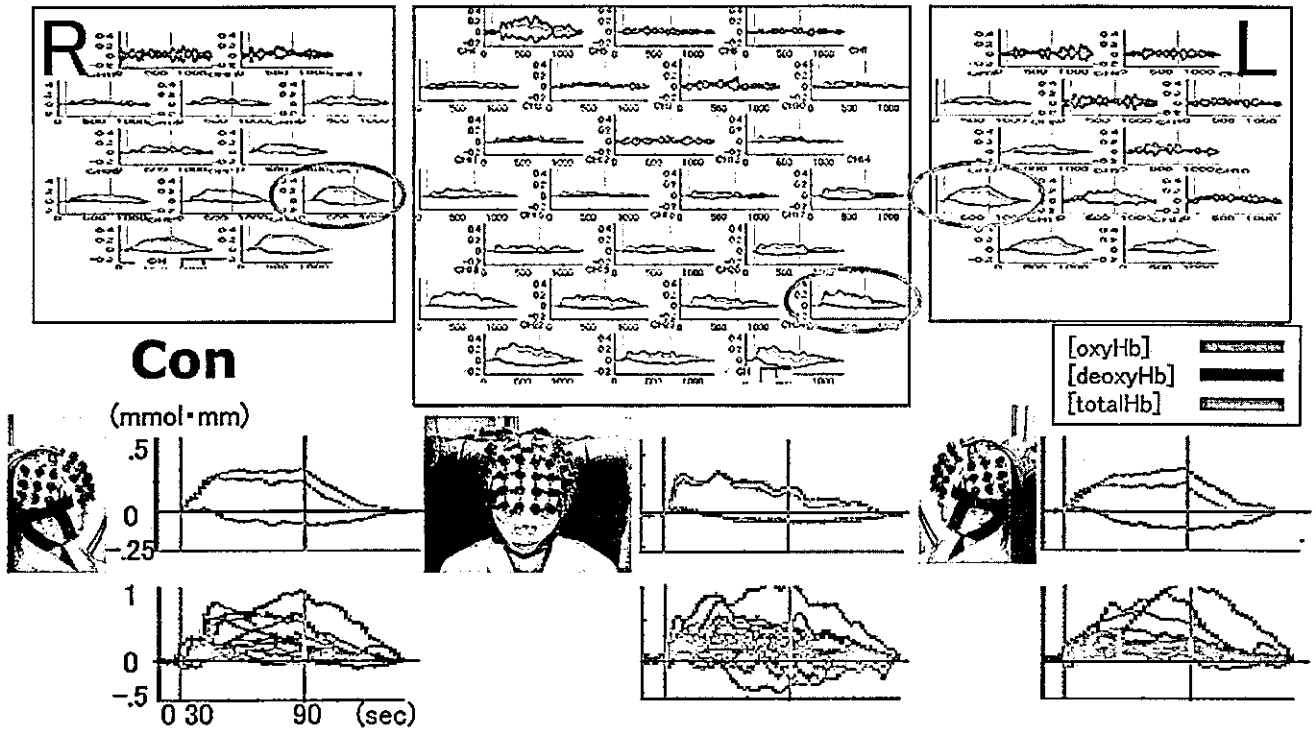

Figure 2. Grand average waveforms of hemoglobin concentration changes during cognitive activation in the control (Con) group. Grand average waveforms of oxyhemoglobin ([oxyHb]; red line), deoxyhemoglobin ([deoxyHb]; blue line), and total hemoglobin [totalHb]; green line) changes during cognitive activation (between two vertical light-blue lines) in the frontal channels (center), and the left (right) and right temporal channels (left) in the control group. Three sets of grand average waveforms and superimposed individual waveforms of [oxyHb] changes in representative channels (circled in orange) are enlarged below.

The gender ratio and age did not significantly differ among the groups.

All the subjects were right-handed, as based on their Edinburgh scores (Oldfield 1970; mean 95.6, SD 7.9, range 76.5–100). This study was approved by the institutional review board of Gunma University Graduate School of Medicine, and written informed consent was obtained from all the subjects before the study.

Activation Tasks

Hemoglobin concentration changes were measured during cognitive and motor activations. The subjects each sat on a comfortable chair in a daylight room with their eyes open throughout the measurements. The cognitive activation consisted of a 30-sec pretask baseline, a 60-sec word fluency task, and a 60-sec posttask baseline. In the word fluency task, the subjects were instructed to generate as many words whose initial syllable was /a/, /ka/, or /sa/ as they could. The three initial syllables changed in turn every 20 sec during the 60-sec task, to reduce the time during which the subjects were silent. The number of words generated during the word fluency task was determined as a measure of task performance. The subjects were instructed to repeat the syllables /a/, /i/, /u/, /e/, and /o/ during the pretask and posttask baseline periods.

The motor activation consisted of a 30-sec pretask rest, a 40-sec right-finger-tapping task, and a 30-sec posttask rest. The subjects were instructed to tap their four fingers with their thumb in turn as quickly and accurately as they could. They practiced right-finger tapping after receiving instructions, and it was confirmed that they could perform the task correctly.

In both the cognitive and motor activations, the subjects were instructed with an auditory cue at the start and end of the task or baseline period and at the task category change. Between any cognitive and motor activations and the next activation, the subjects had a recess of at least 3 min.

NIRS Measurements

In this study, [oxyHb], [deoxyHb], and [totalHb] were measured with two 24-channel NIRS machines (Hitachi ETG-100; Hitachi Medical Corporation, Tokyo, Japan) at two wavelengths of near-infrared light (760 and 840 nm), the absorption of which was measured, and [oxyHb] and [deoxyHb] were calculated as described by Maki et al (1995). [TotalHb] was calculated as the sum of [oxyHb] and [deoxyHb]. The interprobe distance of the machine was 3.0 cm, and it was determined that the machine measures points 2 to 3 cm beneath the scalp, that is, the surface of the cerebral cortices (Hock et al 1997; Toronov et al 2001).

The probes of the NIRS machines were placed on a subject's frontal and bilateral temporal regions. The probes on the subject's frontal region measured the relative concentrations of Hb changes at 24 measurement points in an 8 × 8 cm area, with the lowest probes positioned along the Fp_1–Fp_2 line, according to the international 10/20 system used in electroencephalography. Each set of probes on the subject's bilateral temporal region measured the relative concentrations of Hb changes at 12 measurement points in a 6 × 6 cm area, with the central probe

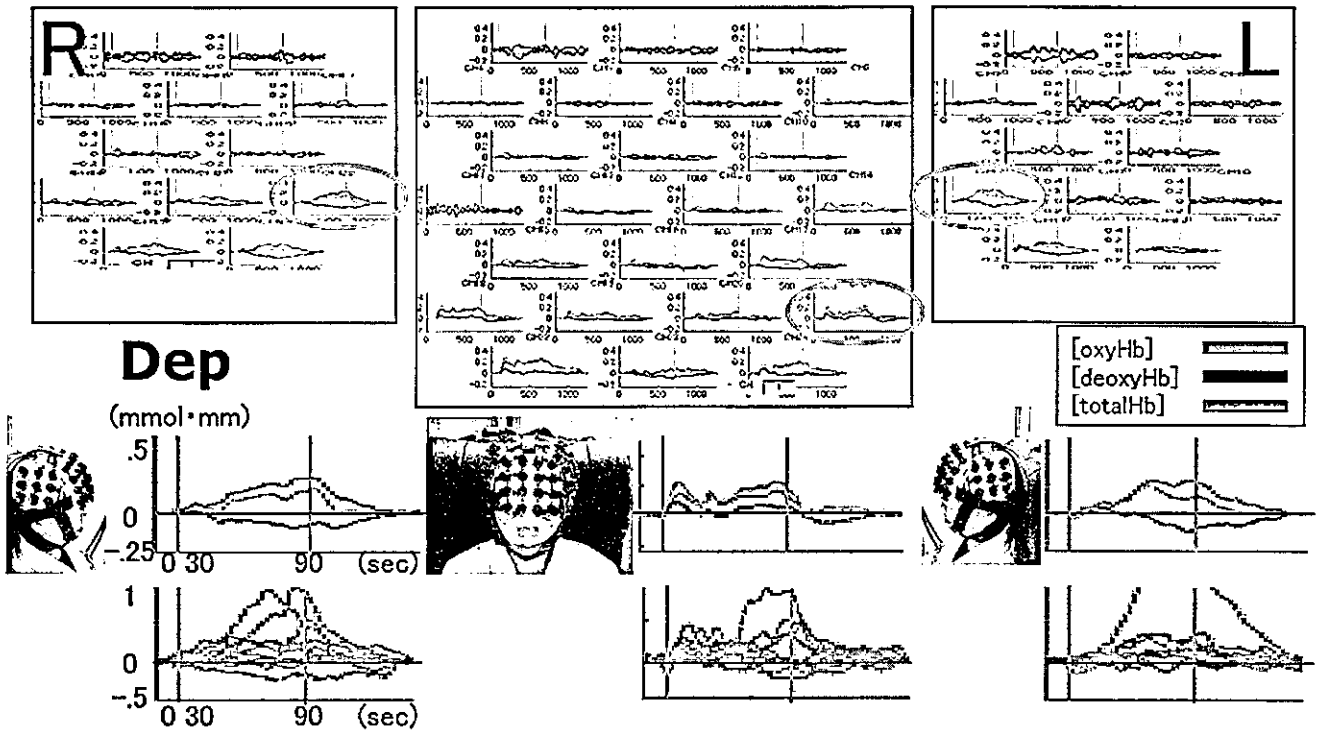

Figure 3. Grand average waveforms of hemoglobin concentration changes during cognitive activation in the depression (Dep) group. Grand average waveforms of oxyhemoglobin ([oxyHb]; red line), deoxyhemoglobin ([deoxyHb]; blue line), and total hemoglobin [totalHb]; green line) changes during cognitive activation (between two vertical light-blue lines) in the frontal channels (center), and the left (right) and right temporal channels (left) in the control group. Three sets of grand average waveforms and superimposed individual waveforms of [oxyHb] changes in representative channels (circled in orange) are enlarged below.

positioned at the midpoint between the vertex and the external ear hole. The correspondence of the probe positions and the measurement points on the cerebral cortex were confirmed by superimposition of the probe positions on a magnetic resonance image of a three-dimensionally reconstructed cerebral cortex of a representative subject in the healthy control group (Figure 1).

The absorption of near-infrared light was measured with a time resolution of .1 sec. The obtained data were analyzed with the "integral mode": the pretask baseline was determined as the mean across 10 sec just before the task period, the posttask baseline was determined as the mean across 5 sec 50 sec (cognitive activation) or 5 across 5 sec 5 sec (motor activation) after the task period, and linear fitting was performed on the data between two baselines. Moving average methods were used to exclude short-term motion artifacts in the analyzed data (moving average window: 5 sec).

According to the above-mentioned measurement parameters for the integral mode, the waveforms of [oxyHb], [deoxyHb], and [totalHb] changes were acquired from all the subjects in all 48 channels during the cognitive and motor activations. We tried to exclude motion artifacts by closely monitoring artifact-evoking body movements, such as neck movements, strong biting, and blinking (identified as most influential in the preliminary artifact-evoking study), and by instructing the subjects to avoid these movements during the NIRS measurements. Moreover, data that clearly contained motion artifacts, determined based both on our observation and on the NIRS recording, were excluded from further analyses.

The grand average waveforms of three types of Hb concentration changes and superimposed individual waveforms of [oxyHb] changes were obtained in all the subjects, based on individual subjects' waveforms in all 48 channels. These grand average waveforms of [oxyHb] changes in all 48 channels were also imaged as topographs of [oxyHb] changes with the linear compensation method.

[OxyHb] data in the three groups were compared in two ways. First, as is usually done in block design experiments, [oxyHb] data were averaged across three task segments (pretask, task, and posttask), analyzed with two-way analysis of variance (ANOVA) with "diagnosis" (control, depression, and schizophrenia) and "task segments" (pretask, task, and posttask) as independent variables in all 48 channels, and compared by post hoc Scheffe multiple comparison. Second, [oxyHb] changes were compared between each of the two patient groups and the control group with Student *t* tests using the grand average waveforms every .1 sec in each channel. This analysis enabled more detailed comparison of [oxyHb] changes along the time course of the task. Significance level corrections for multiple comparisons were not carried out in this analysis.

Results

Cognitive Activation

The number of words generated during the word fluency task showed no statistically significant differences among the three groups (depression group: mean 14.6, SD 5.7; schizophrenia

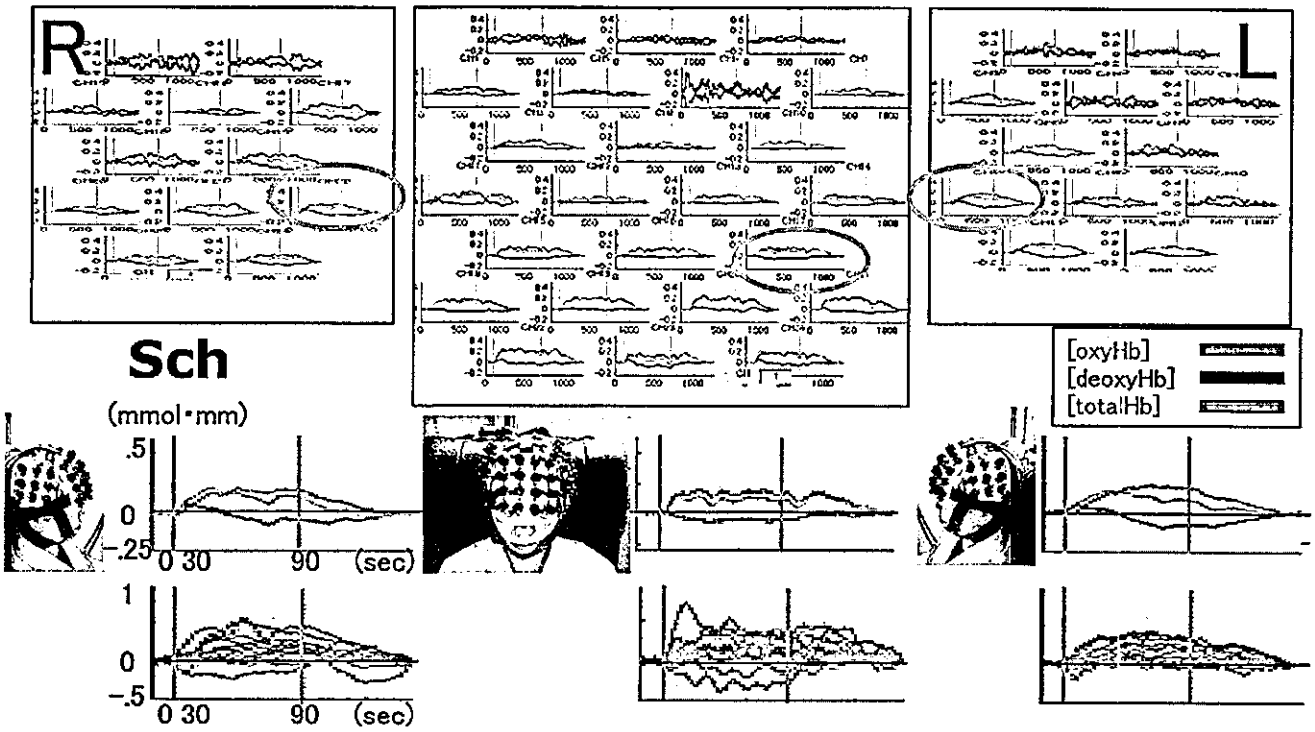

Figure 4. Grand average waveforms of hemoglobin concentration changes during cognitive activation in the schizophrenia (Sch) group. Grand average waveforms of oxyhemoglobin ([oxyHb]; red line), deoxyhemoglobin ([deoxyHb]; blue line), and total hemoglobin [totalHb]; green line) changes during cognitive activation (between two vertical light-blue lines) in the frontal channels (center), and the left (right) and right temporal channels (left) in the control group. Three sets of grand average waveforms and superimposed individual waveforms of [oxyHb] changes in representative channels (circled in orange) are enlarged below.

group: mean 15.1, SD 3.5; control group: mean 16.8, SD 3.6; comparison among the three groups: $F = 1.04$, $p = .36$; one-way ANOVA).

Figures 2–4 show the grand average waveforms of [oxyHb], [deoxyHb], and [totalHb] during the cognitive activation in the control, depression, and schizophrenia groups, respectively. The three sets of waveforms of Hb changes enlarged below were those in the representative channels in which the difference in [oxyHb] changes between the patients and control subjects was most statistically significant. The superimposed individual waveforms of [oxyHb] changes shown at the bottom of the figures confirmed that the grand average waveforms essentially represent most individual data in each group. Figure 5 shows the topographs of [oxyHb] changes for the three groups during the cognitive activation. As shown in the grand average waveforms, Hb concentrations could not be measured

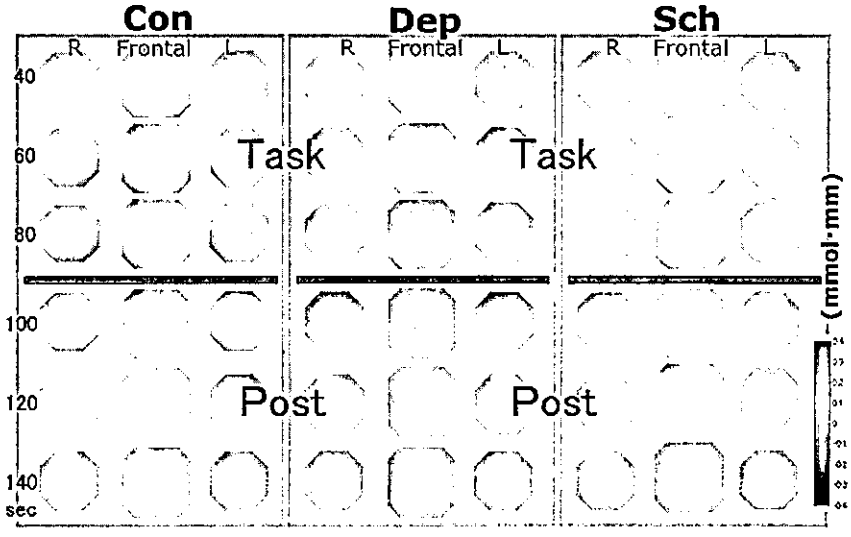

Figure 5. Topographic presentation of oxyhemoglobin [oxyHb] changes during cognitive activation in the three groups. The [oxyHb] changes in the control (Con, left), depression (Dep, center), and schizophrenia groups (Sch, right) are presented as topographic maps along the time course of the task (from top to bottom). Each set of topographic maps is composed of three maps corresponding to the results in the frontal and bilateral temporal channels. The time from the start of the task is presented in seconds on the left. The red, green, and blue areas in the topographs indicate increase, no change, and decrease in [oxyHb], respectively.

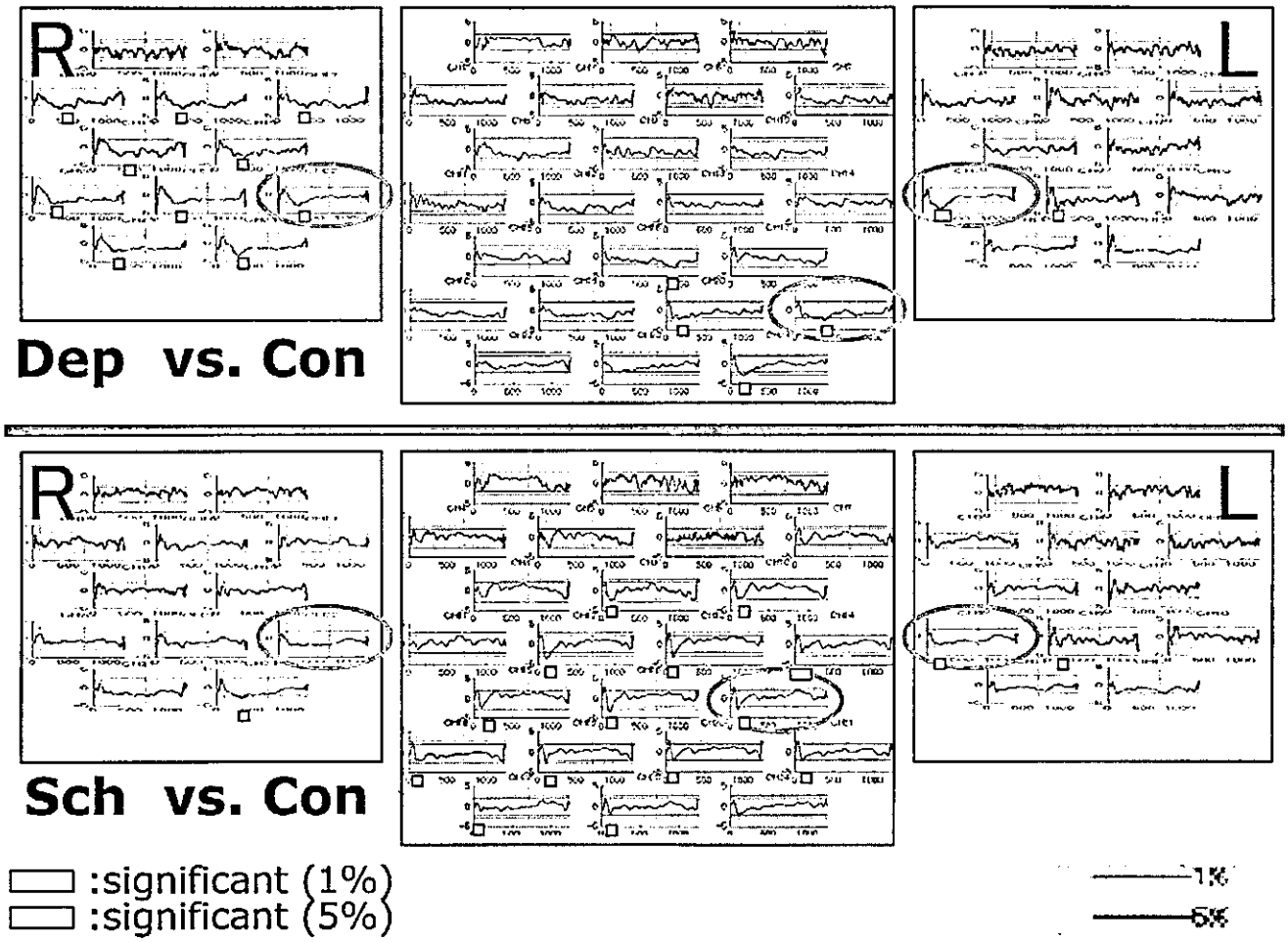

Figure 6. *t* value graphs showing oxyhemoglobin [oxyHb] comparison between the patient and control groups during cognitive activation. *t* values of [oxyHb] comparison between the depression and control groups (Dep vs. Con, top), and between the schizophrenic and control groups (Sch vs. Con, bottom) in 48 channels as presented as in Figure 2. Blue and red lines in each *t* graph correspond to 5% and 1% statistical significance levels, respectively, and the times with significant differences in each graph are marked light yellow and yellow.

with a sufficient signal/noise ratio in the upper frontal and temporal channels.

In the control group (Figure 2), clear [oxyHb] increases were observed during the task period, and the time-course patterns of the changes differed depending on the measuring channel. In the frontal channels, [oxyHb] rapidly increased immediately after the start of the task period, was maintained at the activated level during the task period, and decreased gradually after the task was finished. In the temporal channels, [oxyHb] continued to increase across the task period, peaked at the end of the task period, and decreased gradually in the posttask period. Such [oxyHb] increases during the task period were clearly observed in the lower frontal and anterior lower temporal channels (Figure 5).

In the depression group (Figure 3), [oxyHb] increases during the task period were smaller than those in the control group for both the frontal and temporal channels across the task period. The [oxyHb] topographs show that the increases were observed in the lower frontal and anterior lower temporal channels, as in the control group (Figure 5).

In the schizophrenia group (Figure 4), [oxyHb] showed a sustained moderate increase during the task period and began to decrease immediately after the end of the task period, but then re-increased during the posttask period, particularly in the left frontal channels. The [oxyHb] changes were prominent in almost the same regions as those in the control and depression groups (Figure 5). Additionally, detailed examination of the grand average waveforms shown in Figure 4 revealed a small trough of [oxyHb] immediately after the start of the task period in the frontal channels, which was not evident in the control and depression groups.

The two-way ANOVA revealed a significant main effect of "task segments" in 31 of 48 channels, and post hoc comparison confirmed that [oxyHb] increases during the task period were larger than those in the pretask period. The main effect of "diagnosis" was significant in only eight of 48 channels, and post hoc comparison clarified that [oxyHb] increases in the depression group were smaller than those in the control group in two channels and in the schizophrenic group in two other channels. All the interactions of "task segments" and "diagnosis" were nonsignificant.

The results of the *t* test for the between-group comparison of

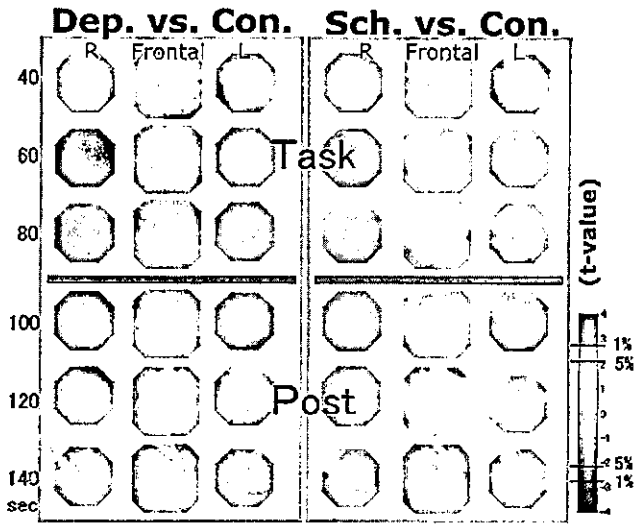

Figure 7. Topographic presentation of *t* value of oxyhemoglobin [oxyHb] comparison between the patient and control groups during cognitive activation. *t* values of [oxyHb] for the depression and control groups (Dep. vs. Con., left) and the schizophrenia and control groups (Sch. vs. Con., right) are presented as topographic maps along the time course of the task (from top to bottom). Each set of topographic maps is composed of three maps corresponding to the results in the frontal and bilateral temporal channels. The time from the start of the task is presented in seconds on the left. The red, green, and blue areas in the topographs indicate positive, zero, and negative *t* values, with 2.1 and 2.8 for 5% and 1% statistical significance levels, respectively.

[oxyHb] changes during the cognitive activation are shown in Figure 6. Parts of the results are shown in Figure 7 in the form of topographs. [OxyHb] increases in the depression group were significantly smaller than those in the control group during the first half of the task period in the left lower frontal ($p < .05$) and bilateral lower anterior temporal channels ($p < .01$).

In the schizophrenia group, [oxyHb] increases were smaller immediately after the start of the task period in the lower frontal channels ($p < .01$) and during the first half of the task period in the bilateral lower anterior temporal channels ($p < .05$), but rather larger for the posttask period in one left frontal channel ($p < .05$) than those in the control group.

Motor Activation

The grand average waveforms of [oxyHb], [deoxyHb], and [totalHb] changes during the finger-tapping task are shown in Figure 8. [OxyHb] increases during the finger tapping task in the depression group were significantly larger than those in the control group during the latter half of the task period in the left frontal channels ($p < .01$, data not shown), and those in the schizophrenic group were not significantly larger than those in the control group. Thus, [oxyHb] increases in the patient groups were or tended to be larger than those in the control group during the finger-tapping task and were smaller during the word fluency task.

Discussion

In this study using multichannel NIRS machines, the temporal and topographic characteristics of rCBV changes in the depressive and schizophrenic patients were compared with those in healthy controls during cognitive and motor activations. [OxyHb] increases during the task period were evident in most NIRS channels during both activations in all three groups. The time course of [oxyHb] increases, however, differed among the three groups with the same performance in the word fluency task: compared with the control group, the major depression group was characterized by a smaller [oxyHb] increase during the first half of the task period in the frontal and temporal channels, and the schizophrenic group by a small trough of [oxyHb] at the start of the task period, by a smaller [oxyHb] increase during the first half of the task period in the temporal channels, and by an [oxyHb] re-increase in the posttask period in one frontal channel. The differences in [oxyHb] increase between the patient and control groups observed in the word fluency task were assumed to be task-specific, because [oxyHb] increases in the patient groups were rather larger than those in the control group during the finger-tapping task.

Three points should be noted regarding the task parameters used in this study. First, the task period was longer in the present study. Most previous NIRS studies using finger-tapping and word fluency tasks used shorter task periods (i.e, 10–30 sec [Colier et al 1999; Maki et al 1995; Obrig et al 1996; Watanabe et al 1998]), although some previous studies used a 60-sec task period (Hirth et al 1997; Hock et al 1997; Matsuo et al 2000, 2002). We adopted longer task periods (40 sec for the finger-tapping task and 60 sec for the word fluency task) to enable a more detailed examination of the time course of rCBV changes. Second, we adopted a modified version of the word fluency task: the beginning syllables of the words to be generated were changed every 20 sec for the 60-sec task period. The reason for the use of this modified version was to avoid a silent period in the task. Some of the subjects, particularly the psychiatric patients, who experienced difficulty in generating words, tended to stop thinking and to become silent during the task period, which resulted in the loss of cerebral activation. Therefore, the syllables assigned to the words were changed after a shorter period to make the task easier. These changes resulted in the lack of significant difference in performance among the three groups. Third, the subjects were required to repeat syllables during the baseline periods in the word fluency task, instead of remaining silent as in other studies. The observed [oxyHb] changes during the task period, therefore, indicate the differences between simple utterance and word fluency tasks. This procedure enables the differentiation between the utterance process and the word-generating effort.

The [oxyHb] increases observed during the task period of the word fluency task and confirmed by ANOVA are assumed to reflect the rCBV increases due to the task-related cortical activation, hence demonstrating that cerebral activation due to cognitive activation is successfully detected by NIRS. The [oxyHb] increase differences between either the patient groups and the control groups were significant in only four channels when [oxyHb] changes were averaged across each time segment; however, when [oxyHb] increases were compared along the time course of the task, as shown in the *t* test results, many more channels showed significant differences, mainly in the lower frontal and anterior lower temporal channels, where the signal/noise ratio was higher. Moreover, the time courses of such significance were also assessed every .1 sec.

[OxyHb] increases in the patients with major depressive disorders were smaller than those in the control subjects during the word fluency task, even though the task performance was not significantly different between the two groups. Significant group differences in cognitive activation were observed in the lower left frontal and bilateral anterior lower temporal channels,

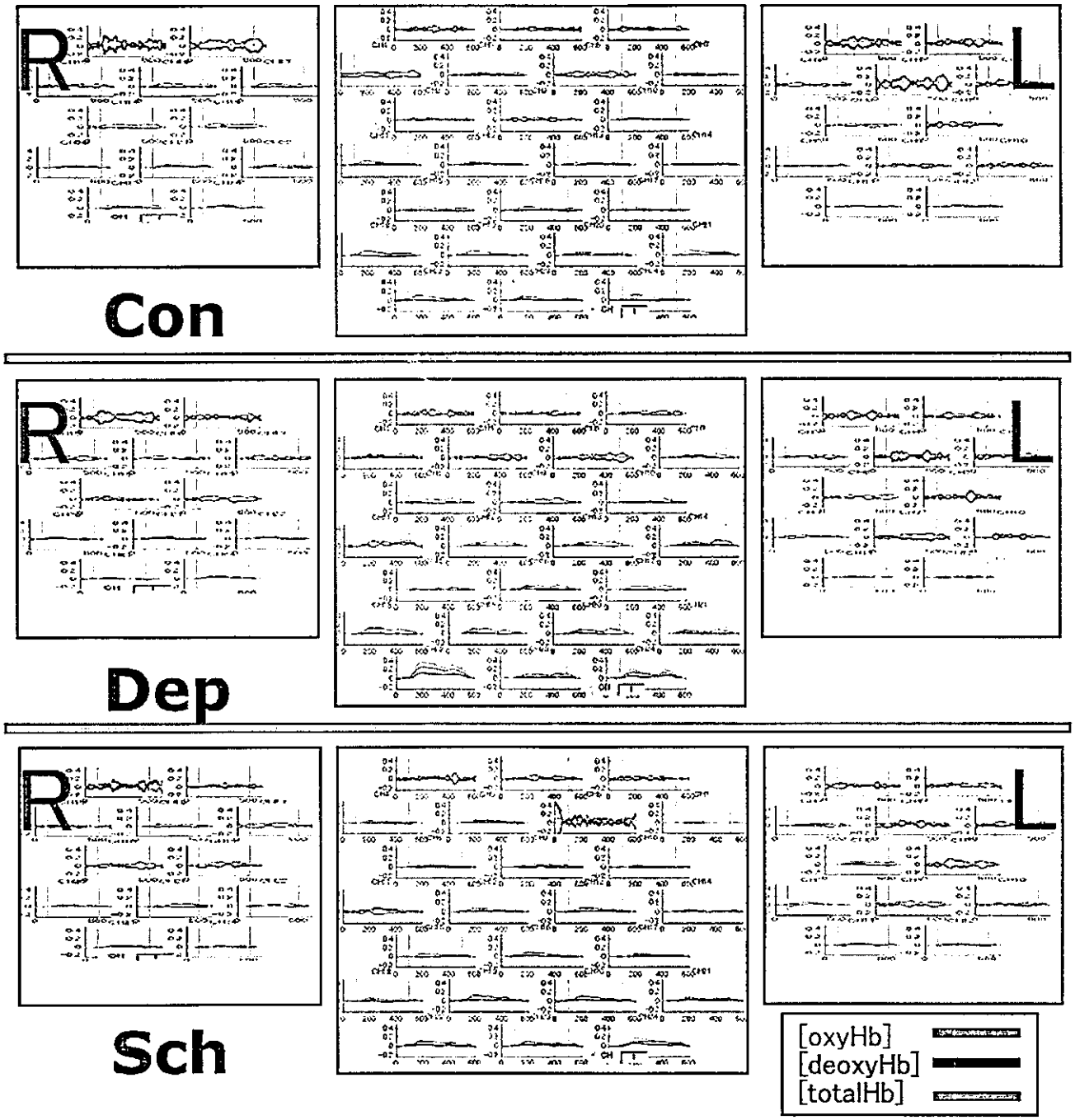

Figure 8. Grand average waveforms of hemoglobin concentration changes during motor activation in the three groups. Grand average waveforms of oxyhemoglobin ([oxyHb]; red line), deoxyhemoglobin ([deoxyHb]; blue line), and total hemoglobin ([totalHb]; green line) during motor activation (between two vertical light blue lines) in the control (Con, upper), depression (Dep, middle), and schizophrenia groups (Sch, lower).

which are assumed to almost correspond to the left lower dorsolateral prefrontal area and the bilateral perisylvian area, respectively. Decreased [oxyHb] activation in depression is consistent with decreased rCBF and metabolism in the dorsolateral prefrontal cortex in the resting state observed in functional neuroimaging studies using other methodologies, such as PET, SPECT, and fMRI, as reviewed by Drevets (2000). The decreased rCBV activation during the cognitive task period in this study indicates that the cerebral cortex of depressive patients cannot gain a sufficient increase in blood supply to overcompensate for the consumed oxygen as in the case with healthy control subjects. The repeated loss of overcompensation for blood supply could result in a shortage of reserved energy in the cerebral cortex and in its clinical presentation as depression.

In contrast to the depression group, no significant differences in [oxyHb] increase during the task period, except a few seconds just after the start of the task, in the frontal channels were found between the schizophrenia and control groups during the word fluency task. The absence of significant differences was not in agreement with either the hypofrontality (Andreasen et al 1998) observed when the task performances of schizophrenic patients are poorer or hyperfrontality (Weinberger et al 2001) observed when the task performances are matched, but it was in agreement with the normal magnitude of frontal activation seen in verbal fluency tasks in some neuroimaging studies (for example, Frith et al 1995). Modified characteristics in the word fluency task used in the present study and nonsignificant difference in performances between the schizophrenic and control groups could partly explain the results. In the bilateral temporal channels, which are assumed to correspond to the perisylvian area, [oxyHb] increases were smaller than those in the control group. This result is consistent with results of previous PET and SPECT studies that showed rCBV reduction in the temporal cortex of schizophrenic patients (Ragland et al 2001; Riehemann et al 2001) and might reflect an impaired function of the temporal cortex.

Other findings in schizophrenia were that the [oxyHb] change difference between the schizophrenic and control subjects were found not during the task period but immediately after the start and end of the task period (i.e., the small trough of [oxyHb] at the start of the task period and the [oxyHb] re-increase in the posttask period, respectively). Both these results suggest the inefficient activation of the prefrontal cortex along the time course of the task demand in schizophrenic patients: for example, insufficient activation at the start of the task and unnecessary activation after the task was finished. Such a detailed examination of the rCBV change pattern along the time course was made possible by the high time resolution of the NIRS machine.

The cognitive task specificity associated with the above results should be noted. [OxyHb] increases in the frontal channels in the patient groups were smaller in the word fluency task but were larger in the finger-tapping task than those in the control group. These differences between the tasks suggest that the obtained findings cannot be explained by general and nonspecific factors, such as impaired vascular responsiveness irrespective of neural activation, but by the difference in neural activation in the frontal cortex. The altered activations of the frontal channels observed in the present study, therefore, are assumed to reflect cerebral blood flow aspects of the frontal lobe dysfunction in depression and schizophrenia.

The NIRS methodology used in the present study has limitations: NIRS enables measurement of Hb concentration changes 1) only as relative values but not as absolute values; 2) only in the cortex immediately beneath the probes but not in deeper brain structures; 3) with a high time resolution but with a poor spatial resolution in imaging methodologies for cerebral blood flow and metabolism; and 4) not only in the brain but also in more surface structures, such as the skin and skull. We examined the influence of the first limitation by superimposing the individual waveforms of [oxyHb] changes in each group, and found essentially similar time courses of [oxyHb] changes among the subjects in each group. These results confirm that summing the individual waveforms of [oxyHb] changes within each group generates a significant grand average waveform. The second and third limitations could be resolved in future studies on simultaneous measurements by NIRS and other neuroimaging methodologies, such as PET, SPECT, and fMRI. The fourth limitation can be resolved if blood volumes in the skin, muscle, and skull are constant. Hence, we monitored the movements of a subject's head and extremities during the experiment and excluded the data contaminated with such artifacts.

There are three points that should be improved in the present study. The first point is the limited cerebral regions that could be measured with NIRS probes. A considerable nonmeasured area existed between the areas covered by the frontal and temporal probes, owing to their arrangement on the skull, which prevented the examination of the lower posterior frontal cortex. The second point is the subjects' characteristics. The sample sizes were small, the gender ratios were somewhat skewed, and the patients' symptoms were rather mild. The third point is that all the patients were taking medications at the time of the examination, and it is possible that at least part of the observed findings could result from the effects of these psychotropic drugs. Further studies are needed to improve on these three points.

In conclusion, the characteristic patterns of [oxyHb] changes in the frontal lobe were assessed for depression and schizophrenia in this study. The findings coincided with the proposed frontal lobe dysfunction in depression and schizophrenia determined by PET, SPECT, and fMRI and clarified in more detail the dysfunction along the time course of [oxyHb], owing to the high time resolution of the NIRS machine. Near-infrared spectroscopy, with its noninvasiveness and high time resolution, could be a useful research tool for the examination of brain functions in psychiatric disorders when combined with other methodologies, as well as a clinically useful tool for the diagnosis and treatment of individual psychiatric patients in the near future.

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Genetic variations in the WFS1 gene in Japanese with type 2 diabetes and bipolar disorder

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Abstract

Diabetic and psychiatric symptoms often appear in patients with Wolfram syndrome, and obligate carriers of *WFS1* have increased prevalence of type 2 diabetes and are more likely to require hospitalization for psychiatric illness including bipolar disorder. To identify the polymorphisms in Japanese, we examined a region of ~50 kb covering the entire *WFS1* gene, and evaluated the patterns of linkage disequilibrium. We found a total of 42 variations including 8 novel coding single nucleotide polymorphisms (A6T, A134A, N159N, T170T, E237K, R383C, V412L, and V503G), 14 novel non-coding polymorphisms, and 2 linkage disequilibrium blocks. We also performed association studies in patients with type 2 diabetes mellitus and patients with bipolar disorder. The haplotype comprising R456 and H611 was most associated with type 2 diabetes ($p = 0.013$) and the haplotype comprising g. −15503C/T and g. 16226G/A was most associated with bipolar disorder ($p = 0.006$), but neither reached significant difference after multiple adjustment. These genetic variations and linkage disequilibrium patterns in *WFS1* in Japanese should be useful in further investigation of genetic diversities of *WFS1* and various related disorders.

Keywords: Genetics; Mood disorder; Population study; Linkage disequilibrium; Single nucleotide polymorphism

Introduction

Wolfram syndrome, also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness [OMIM 222300]), was first described by Wolfram and Wagener [1]. While only juvenile onset diabetes mellitus and progressive optic nerve atrophy are required for the diagnosis, many patients also develop diabetes insipidus, sensorineuronal hearing loss, ataxia, peripheral neuropathy, urinary tract atonia, and psychiatric illnesses such as psychosis, severe depression, and dementia [2].

Wolfram syndrome has been shown to have links with D4S432–D4S431 at chromosome 4p16 [3,4]. We earlier reported two siblings with Wolfram syndrome who demonstrated mood symptoms [5], and proceeded with a multi-institutional coordinated effort to discover the genetic etiology of the disease. Recently, mutations in the gene *WFS1/wolframin* were identified in patients with Wolfram syndrome [6,7]. The gene, which encodes a novel protein containing the predicted transmembrane domains, is expressed ubiquitously, with high expression in pancreatic islets and specific neurons (hippocampus CA1, amygdaloid areas, olfactory tubercles, and superficial layers of the allocortex). The subcellular localization of this protein has been determined to be primarily in the endoplasmic reticulum [8], but it's function is not established.

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The prevalence of this autosomal recessive syndrome was estimated as ~1/770,000 in the United Kingdom [9]. While Wolfram syndrome is rare, obligate carriers have increased prevalence of type 2 diabetes mellitus [9,10], and heterozygous carriers are reported to be 26-fold more likely to require hospitalization for psychiatric illness [11].

Bipolar disorder, also called manic-depression, is characterized by mood swings between states of depression and elation, and genetic predisposition is thought to be an important factor. A linkage study of a large Scottish family provided a maximum LOD score of 4.8 in the region D4S431–D4S403 [12]. Other groups have presented supportive linkage evidence with markers in this region [13,14]. The psychiatric phenotypes observed in carriers and patients with Wolfram syndrome and the location of *WFS1* in the region linked to bipolar disorder have suggested a role of the gene in the development of the disease.

Evidence of abnormal glucose metabolism in psychiatric patients has been accumulating since the early 20th century [15]. Some studies report an increased prevalence of diabetes in hospitalized manic-depressives [16–19]. Gavard et al. carried out an evaluation of 20 studies of the diabetic personality, and found an increased prevalence of depression. A relationship between psychiatric disorder and diabetes mellitus has been suggested by mutations in *WFS1* that affect both diabetic and psychiatric phenotypes. Indeed, we estimated the LOD score for susceptibility to type 2 diabetes in one of the Wolfram pedigrees (WS-1) [6] using additional family information, and found suggestive linkage (M. Mikuni et al. unpublished).

In this study, we examined all of the regions of *WFS1* in Japanese to detect single nucleotide polymorphisms (SNPs)[1] as genetic markers, and evaluated the pattern of linkage disequilibrium (LD) to provide information on population diversity in this gene. We also performed association studies in Japanese patients with type 2 diabetes mellitus and patients with bipolar disorder.

Materials and methods

Subjects

One hundred and ninety two patients with type 2 diabetes mellitus (male/female, 114/78; age, 62.0 ± 11.2 years; age at diagnosis, 49.8 ± 11.0 years; postprandial glucose, 168.5 ± 69.0 mg/dl; hemoglobin (Hb) A_{1C}, 6.7 ± 1.1%; body mass index (BMI), 23.9 ± 3.5 kg/m^2) and 192 controls (male/female, 74/118; age, 67.6 ± 5.8; HbA_{1C}, 4.9 ± 0.3%; BMI, 22.9 ± 2.7 kg/m^2) were examined. Patients were diagnosed with type 2 diabetes by medical records or by 75 g oral glucose tolerance test according to the criteria of the Japan Diabetes Society. Control subjects were recruited on the following criteria: 60 or more years of age, no past history of diagnosis of diabetes, HbA_{1C} less than 5.6%, and no diabetes in family members or second degree relatives. Eighteen patients with bipolar I disorders (male/female, 9/9; age, 46.7 ± 12.4) and 29 patients with bipolar II disorders (male/female, 16/13; age, 53.6 ± 12.3) were recruited from Gunma University Hospital and local hospitals in Gunma prefecture, met DSM-IV diagnostic criteria (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) [20], and were assessed by trained clinicians on the basis of unstructured interviews supplemented by case-note reviews. Ninety-six Japanese random controls (male/female, 39/57; age, 68.8 ± 5.6) were examined for comparisons of genetic variations. The study was approved by the Ethics Committee of Gunma University, and included the written informed consent of each subject.

Detection of polymorphisms in WFS1

Genomic DNA was extracted from samples of whole blood using QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Twelve of the random control samples (24 alleles) were used to detect single nucleotide polymorphisms (SNPs) in *WFS1*. Primers for PCR experiments were designed by Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) on the basis of the genomic contig sequence (GenBank Accession No. NT_006051) of the *WFS1* region. The mixture for the PCR was 20 μl in 10 ng template DNA, 0.5 mM each dNTP, 2.5 pmol each forward and reverse primer, 0.5U Ex*Taq* polymerase (Takara, Kyoto, Japan), and 2 μl of 10× PCR buffer. The reaction conditions were an initial denaturation step of 95 °C for 3 min, subsequent 40 cycle reactions at 94 °C for 30 s, 52–62 °C for 30 s, and 72 °C for 1 min, and a final extension step of 72 °C for 10 min. A 3 μl aliquot from each reaction was assayed on a 1% agarose gel to confirm the product, and the remainder was purified using MultiScreen Filtration System (MILLIPORE, Billerica, MA, USA) with Sephadex G-75 (Amersham Biosciences, Piscataway, NJ, USA). Each PCR product was subjected to cycle sequencing with BigDye terminator Cycle Sequencing FS (Applied Biosystems, Foster, CA, USA) using each forward and reverse primer. Reaction products were purified by ethanol precipitation, and sequenced by ABI PRISM 377 sequencer. Results were processed with Autoassembler, version 2.1 (Applied Biosystems, Foster, CA, USA) to compare sequences.

[1] *Abbreviations used:* bp, base pair; SNPs, single nucleotide polymorphisms; LD, linkage disequilibrium; PCR, polymerase chain reaction; cSNPs, coding single nucleotide polymorphisms.

Mutation screening and genotyping of frequent polymorphisms in WFS1

We examined the coding region of *WFS1* and genotyped sixteen frequent SNPs in the 47 bipolar patients and 96 control subjects. All exons were examined in the 192 type 2 diabetic patients and 192 controls.

Estimation of haplotype frequencies and evaluation of pattern of linkage disequilibrium

Haplotypes were inferred by the expectation-maximization method by Arlequin Software (http://anthro.unige.ch/arlequin). The coefficient for LD, D', and r^2 value was estimated by GOLD software (http://www.well.ox.ac.uk/asthma/GOLD).

Statistical analyses

Statistical difference in allele frequencies between bipolar disorder or diabetes and control groups was assessed by χ^2 test (including Fisher's test when one sample number was less than five for a corresponding 2×2 table). Statistical analysis was performed with StatView 5.0 software (SAS Institute, Cary, NC).

Results

Identification of polymorphisms in WFS1

Twelve of the random controls were examined to detect genetic variations in the entire region of *WFS1*, and a total of 42 polymorphisms were identified in this study as shown in Fig. 1 and Table 1. Comparing our data with the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html), 22 of the SNPs are novel. The distribution of polymorphisms was approximately 1/1000 bp in the 49.2 kb of DNA examined.

Evaluation of the pattern of linkage disequilibrium

As shown in Fig. 2, 16 SNPs were used to define haplotypes and to evaluate the pattern of LD. The other SNPs were excluded because of the rarity of minor alleles. As shown in Fig. 2, there are two LD blocks in this region, one ranging from position g. −15503 to g. 14909 and the other from position g. 16226 to g. 25103. The two SNPs at position g. 16226 and g. 16568, and the four SNPs at position g. 19460, g. 20758, g. 23707, and g. 25103 are in complete linkage disequilibrium.

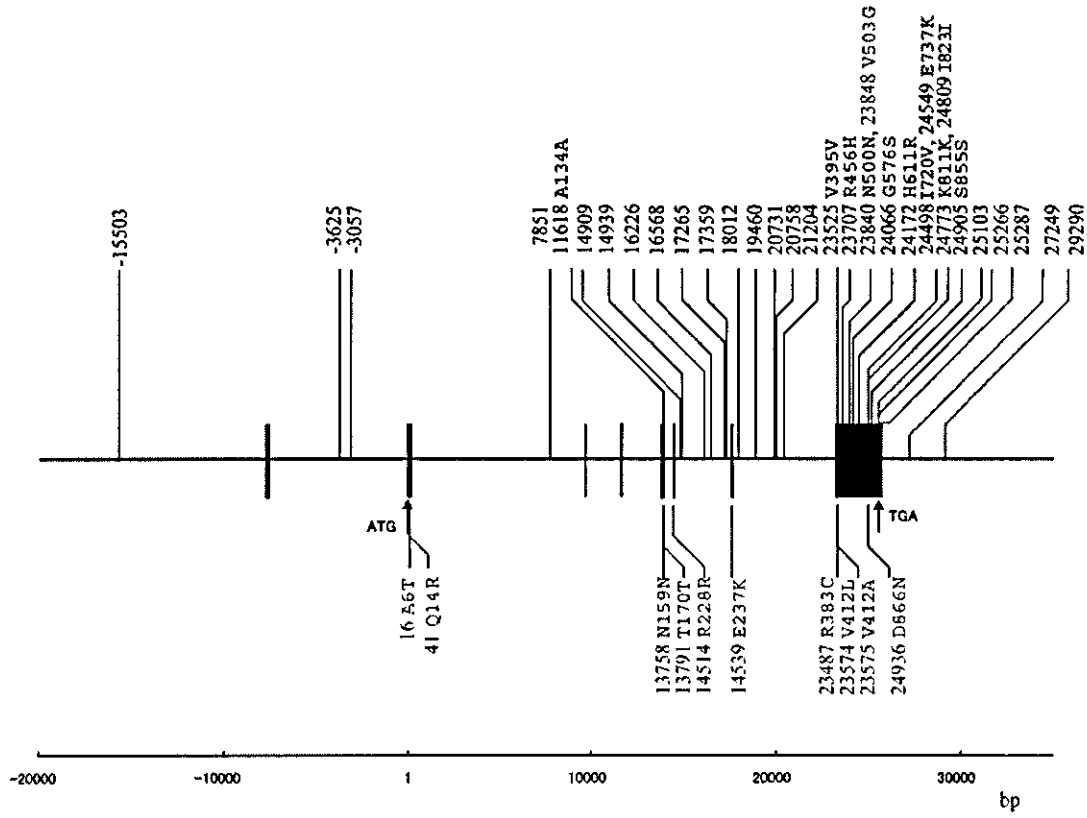

Fig. 1. Polymorphisms of *WFS1* identified in this study. The locations of the polymorphisms described in the text are shown. The nucleotide indicates the location of the SNP relative to the A of the ATG of the initiator Met of *WFS1* (GenBank No. NT_006051). The cSNPs shown in red are observed only in Type 2 diabetic patients. The cSNP shown in blue is observed only in patients with bipolar disorder.

Table 1
Polymorphisms identified in *WFS1* region in this study

Position genome	AA change	Variation	Location	Frequency of minor allele
−15503		C>T*	5′ flanking	0.42
−3625		C>T*	Intron 1	0.21
−3057		G>A	Intron 1	0.46
16	A6T	G>A*	Exon 2	—
41	Q14R	A>G	Exon 2	0.0026
7851		A>G	Intron 2	0.29
11618	A134A	G>A*	Exon 4	0.010
13758	N159N	C>T*	Exon 5	—
13791	T170T	C>G*	Exon 5	0.0079
14514	R228R	G>C	Exon 6	0.010
14539	E237K	G>A*	Exon 7	—
14909		G>A*	Intron 6	0.29
14939		T>C*	Intron 6	0.083
16226		G>A*	Intron 6	0.13
16568		G>A	Intron 6	0.13
17265		G>T*	Intron 6	0.13
17359		C>T*	Intron 6	0.042
18012		G>A*	Intron 7	0.13
19460		G>A*	Intron 7	0.13
20731		C>T	Intron 7	0.29
20758		T>C*	Intron 7	0.13
21204		delCTCA*	Intron 7	0.083
23487	R383C	C>T*	Exon 8	—
23525	V395V	T>C	Exon 8	0.010
23574	V412L	G>C*	Exon 8	0.0026
23575	V412A	T>C	Exon 8	0.0026
23707	R456H	G>A	Exon 8	0.078
23840	N500N	T>C	Exon 8	0.010
23848	V503G	T>G*	Exon 8	—
24066	G576S	G>A	Exon 8	0.12
24172	H611R	A>G	Exon 8	0.094
24498	I720V	A>G	Exon 8	0.063
24549	E737K	G>A	Exon 8	0.047
24773	K811K	A>G	Exon 8	0.010
24809	I823I	C>T	Exon 8	0.005
24905	S855S	G>A	Exon 8	0.010
24936	D866N	G>A	Exon 8	0.0052
25103		G>A*	3′ UTR	0.13
25266		G>A	3′ UTR	0.042
25287		GA	3′ UTR	0.042
27249		delCT*	3′ flanking	0.042
29290		C>T*	3′ flanking	0.13

The nucleotide indicates the location of the SNP relative to the A of the ATG of the initiator Met of *WFS1* (GenBank No. NT_006051). The frequencies of minor alleles of non-coding SNPs shown in this table are observed in random control samples. The frequencies of minor alleles of coding SNPs are observed in 192 non diabetic controls. Asterisk indicates a novel polymorphism.

Association study of genetic variations of WFS1 in patients with type-2 diabetes

All exons were examined in 192 type 2 diabetic patients. We found a total of 21 cSNPs, ten silent mutations and eleven missense mutations, of which seven are novel cSNPs (A6T, A134A, N159N, T170T, E237K, R383C, and V412L). As shown in Table 2, minor alleles H456 and R611were present more frequently in type 2 diabetic patients than in control subjects ($p = 0.091$ and $p = 0.050$, respectively). Because these two cSNPs are in strong linkage disequilibrium, as shown in Fig. 2, the haplotype defined by these SNPs was investigated for association with type 2 diabetes mellitus. The R456–H611 haplotype was less frequent in type 2 diabetic patients than in control subjects (Table 3, $p = 0.013$, $1 - \beta \approx 0.4$), but when we compared the two groups with and without this haplotype, there were no significant differences in age, BMI, fasting and postprandial glucose, or HbA_{1C} (data not shown).

Association study of genetic variations of WFS1 in patients with bipolar disorder

Mutation screening of *WFS1* in 47 patients with bipolar disorders revealed twelve coding SNPs. The allelic frequencies in patients and controls are shown in Table 4. One SNP (c. 402G>A, A134A) was located in exon 4

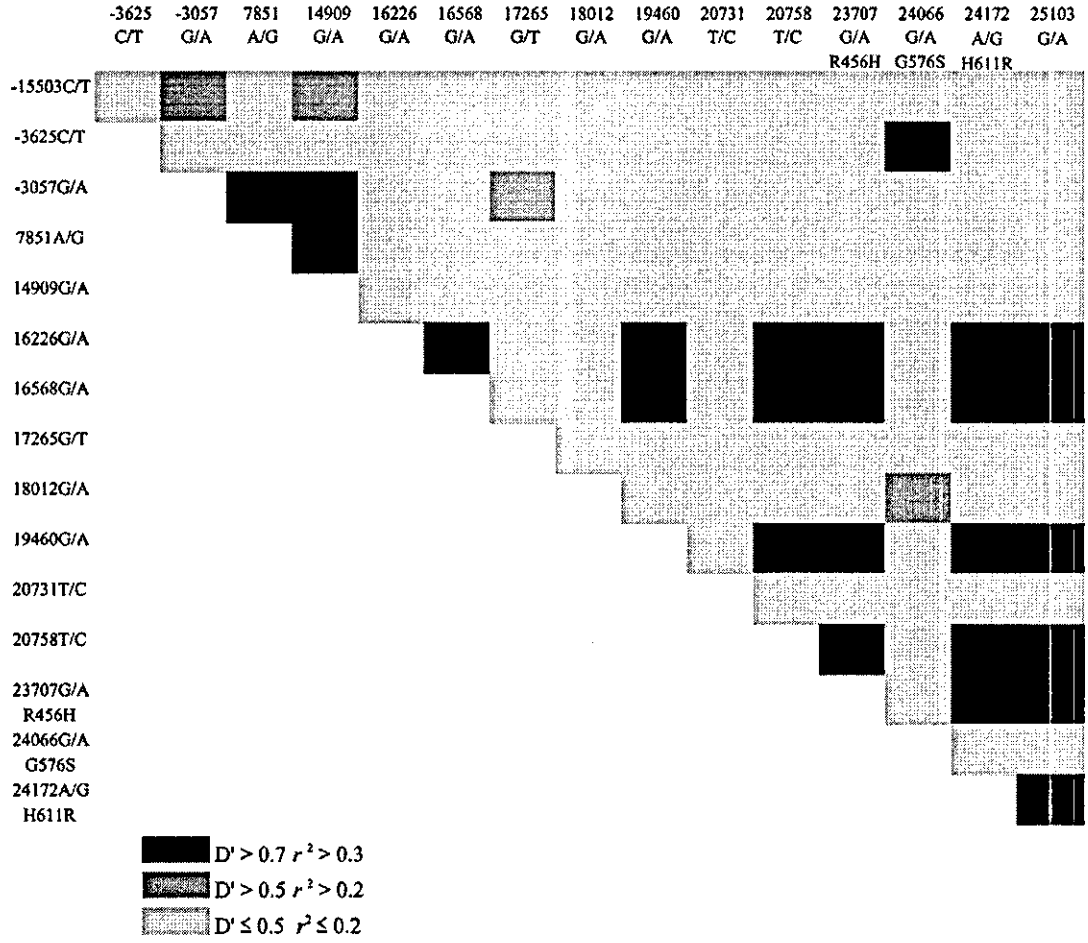

Fig. 2. Pairwise linkage disequilibrium in *WFS1* evaluated by D' and r^2. Extent of pairwise LD of *WFS1*, measured by two distinct coefficients, D' and r^2. Pairwise combinations are classified into three categories based on the degree of the observed LD. Pairwise combination with LD of $D' > 0.7$ and $r^2 > 0.3$, $D' > 0.5$ and $r^2 > 0.2$, and $D' \leqslant 0.5$ and $r^2 \leqslant 0.2$ is shown with black, dark grey, and grey box, respectively. The nucleotide indicates the location of the SNP relative to the A of the ATG of the initiator Met of *WFS1* (GenBank No. NT_006051).

and the others in exon 8. Of the cSNPs identified in this study, two (c. 402G>A, A134A; c. 1508T>G, V503G) were novel and not registered in the NCBI dbSNP database. None of the cSNPs were associated with bipolar disorder, but a novel cSNP (V503G) including four reported cSNPs (V395V, N500N, K811K, and S855S) was observed only in patients with bipolar disorder in a heterozygous state. Pairwise haplotype analysis was performed with combinations of eleven SNPs based on LD pattern (Fig. 3). The haplotype comprising g. −15503C/T and g. 16226G/A is most associated with bipolar disorder ($p = 0.006$), but does not reach significance after multiple adjustment Fig. 3. Association study with an increased number of samples is required.

Discussion

While Wolfram syndrome is rare, obligate carriers show increased prevalence of type 2 diabetes mellitus [9,10], and heterozygous carriers are reported to be 26-fold more likely to require hospitalization for psychiatric illness [11]. A relationship between psychiatric disorder and diabetes mellitus is suggested by mutations in *WFS1* that are observed in both diabetic and psychiatric phenotypes.

We estimated the LOD score for susceptibility to type 2 diabetes in one of the Wolfram pedigrees available and obtained suggestive maximum scores 1.20 and 2.67 at $\theta = 0$ for the dominant and the nonparametric model, respectively (unpublished), leading us to examine all exons of *WFS1* in type 2 diabetes. Ten cSNPs (A6T, Q14R, N159N, T170T, R228R, E237K, R383C, V412L, V412A, and D866N) were found only in patients with type 2 diabetes and not in those with bipolar disorder. Of these, seven cSNPs (A6T, A134A, N159N, T170T, E237K, R383C, and V412L) have not been reported previously [21]. This study shows that the minor alleles H456 and R611 are present more frequently in type 2 diabetic patients than in control subjects, while the

Table 2
Frequencies of coding SNPs in *WFS1* in patients with type 2 diabetes and controls

SNP	Amino acid change	Frequencies of minor allele		*P* value
		Patients ($n = 384$)	Controls ($n = 384$)	
g. 16 G>A	A6T*	0.0027	—	0.49
g. 41 A>G	Q14R*	0.0027	0.0026	>0.99
g. 11618 G>A	A134A*	0.019	0.0086	0.34
g. 13758 C>T	N159N*	0.0027	—	0.49
g. 13791 C>G	T170T*	0.013	0.0079	0.50
g. 14514 G>C	R228R	0.019	0.010	0.38
g. 14539 G>A	E237K*	0.0053	—	0.25
g. 23487 C>T	R383C*	0.0027	—	0.49
g. 23525 T>C	V395V	0.0054	0.0079	>0.99
g. 23574 G>C	V412L*	0.0081	0.0026	0.37
g. 23575 T>C	V412A	0.0054	0.0026	0.62
g. 23707 G>A	R456H	0.12	0.080	0.091
g. 23840 T>C	N500N	0.017	0.0079	0.33
g. 24066 G>A	G576S	0.087	0.11	>0.99
g. 24172 A>G	H611R	0.15	0.10	0.050
g. 24498 A>G	I720V	0.063	0.060	0.87
g. 24549 G>A	E737K	0.049	0.065	0.35
g. 24773 A>G	K811K	0.020	0.0079	0.21
g. 24809 C>T	I823I	0.0085	0.0026	0.73
g. 24905 G>A	S855S	0.017	0.0026	0.53
g. 24936 G>A	D866N	0.011	0.0052	0.44

The nucleotide indicates the location of the SNP relative to the A of the ATG of the initiator Met of *WFS1* (GenBank No. NT_006051). Asterisk indicates a novel polymorphism.

Table 3
Frequencies of haplotypes comprising R456H and H611R in patients with type 2 diabetes and controls

Haplotype	DM	Controls	χ^2	*P* value
R–H	0.83	0.89	6.206	0.013
R–R	0.04	0.03	1.334	0.248
H–H	0.01	0.00	—	0.069
H–R	0.12	0.08	2.207	0.137
	—	—	8.658	0.034

R–H in haplotype column is R456–H611 haplotype.

Table 4
Frequencies of coding-SNPs of *WFS1* in patients with bipolar disorder and in controls

Position genome	Position cDNA	Nucleotide change	Amino acid change	Exon	Frequencies of rare allele		*P* value
					Patients ($n = 94$)	Controls ($n = 192$)	
11618	402	G>A	A134A*	4	0.01	0.01	>0.999
23525	1185	T>C	V395V	8	0.01	0.00	0.33
23707	1367	G>A	R456H	8	0.07	0.08	0.91
23840	1500	T>C	N500N	8	0.01	0.00	0.33
23848	1508	T>G	V503G*	8	0.01	0.00	0.33
24066	1726	G>A	G576S	8	0.13	0.12	0.85
24172	1832	A>G	H611R	8	0.04	0.09	0.16
24498	2158	A>G	I720V	8	0.03	0.06	0.40
24549	2209	G>A	E737K	8	0.03	0.05	0.76
24773	2433	A>G	K811K	8	0.01	0.00	0.33
24809	2469	C>T	I823I	8	0.01	0.01	0.55
24905	2565	G>A	S855S	8	0.01	0.00	0.33

The nucleotide indicates the location of the SNP relative to the A of the ATG of the initiator Met of *WFS1* (GenBank No. NT_006051 for genome, AF 084481 for cDNA); asterisk indicates a novel polymorphism.

R456–H611 haplotype is significantly less frequent and the H456–R611 is more frequent in patients with type 2 diabetes. In the previous study, 370 Japanese patients with type 1 diabetes and 760 control subjects were analyzed, and H456 and R611 were found more frequently in patients than in controls. Preliminary studies in

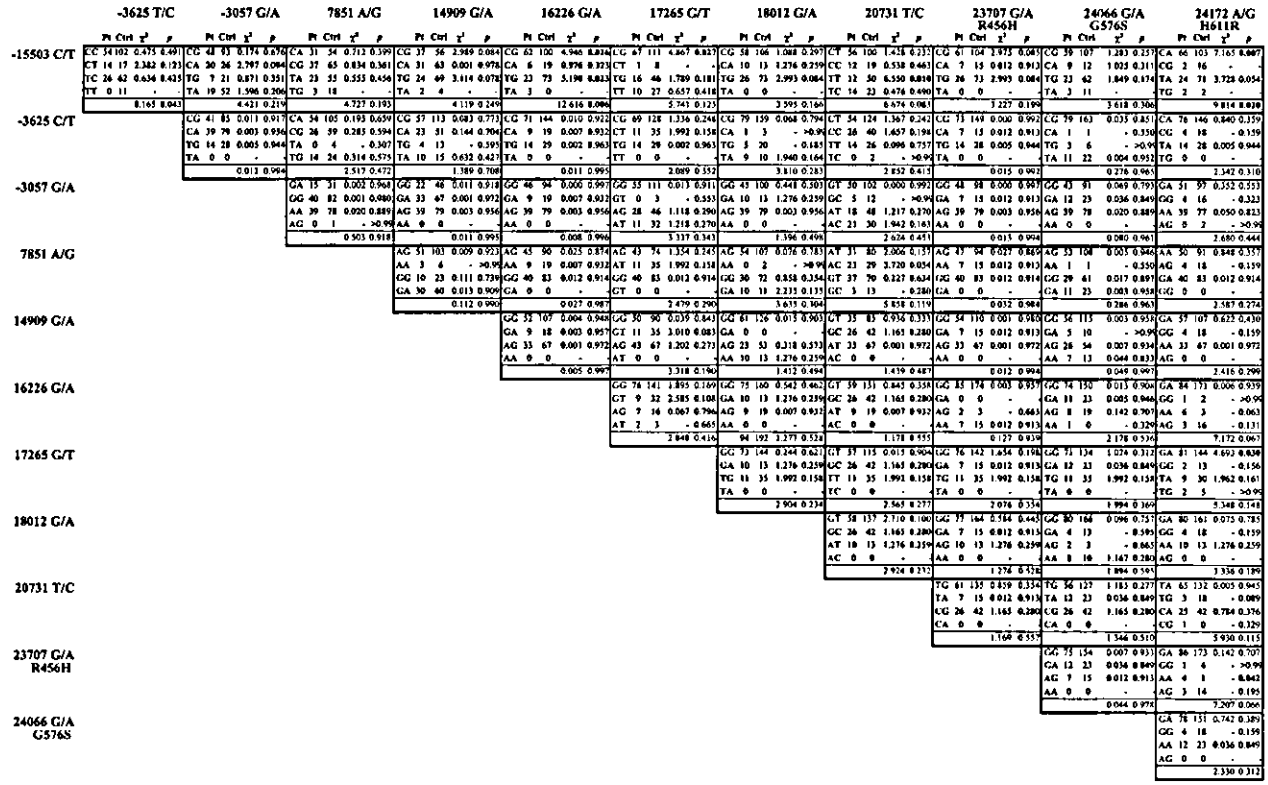

Fig. 3. Association study using pairwise haplotype frequencies in patients with bipolar disorder versus controls. In each cell, P values in the first through fourth row are based on 2×2 χ^2 test, and the P value in the last row is based on 2×4 χ^2 test. P value < 0.05 is indicated in bold.

patients with type 2 diabetes by the same group also showed a significant increase in H456 in type 2 diabetic patients compared to control subjects [22]. These results suggest the protective role of the H456, R611 and their haplotype in the pathogenesis of diabetes. However, the R456–H611 haplotype was found more frequently in type 2 diabetes in the UK [23], suggesting the presence of genetic heterogeneity among populations.

WFS1 is also a candidate for genetic predisposition to bipolar disorder because of the psychiatric phenotypes observed in both carriers and patients with Wolfram syndrome [2]. In addition, *WFS1* is located in a region that has been linked to bipolar disorder [12–14]. Ohtsuki et al. [24] examined exon 8 of *WFS1*, and found no association with bipolar disorder in Japanese. Kato et al. [25] examined four cSNPs (A559T, G576S, A602V, and H611R), and found no association with the disease in Japanese. In this study, we first performed mutation screening of all exons of *WFS1* in 47 Japanese patients with bipolar disorders, and identified 12 coding SNPs (Table 4). Two (c. 402G>A, A134A; c. 1508T>G, V503G) of these were novel, but none were significantly associated with bipolar disorder. To detect all of the polymorphisms in the *WFS1* gene, we examined about 50,000 bp covering the entire region of the gene in 12 Japanese subjects, and identified a total of 42 genetic variations. 18 SNPs and two insertion/deletion polymorphisms were identified in non-coding regions, and 22 SNPs were identified in coding regions. Pairwise haplotype analysis then was performed with combinations of eleven SNPs based on the LD pattern estimated using the 16 frequent SNPs (Fig. 2).

As shown in Fig. 3, we defined haplotypes by all possible pairs of 11 SNPs, and examined the associations with bipolar disorder. The haplotype comprising g. −15503C/T and g. 16226G/A was most associated with bipolar disease ($p = 0.006$), but does not reach significant difference after multiple adjustments. One possible reason for not finding a significant association with bipolar disorder is that bipolar sample in the present study consists mostly of bipolar II patients ($n = 29$) rather than bipolar I patients ($n = 18$). Most studies of genetic linkage in bipolar disorder have samples that are predominantly bipolar I as this syndrome is more well defined. Further study with an increased number of samples is required to determine the contribution of this haplotype to the disease.

In the present study, we have identified a total of 42 polymorphisms as well as the precise LD pattern in *WFS1*. While some of the haplotypes of *WFS1* may be associated with type 2 diabetes or bipolar disorder, the data are inconclusive because of the small number in the study sample. Although the functional properties of the genetic variations in *WFS1* that might affect susceptibility to type 2 diabetes or bipolar disorder are not