Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
International Journal of Psychophysiology 52 (2004) 147–157
INTERNATIONAL JOURNAL OF PSYCHOPHYSIOLOGY
www.elsevier.com/locate/ijpsycho

Correlation between P50 suppression and psychometric schizotypy among non-clinical Japanese subjects

Jijun Wang[a,*], Hiroshi Miyazato[a,b], Hiroto Hokama[a], Ken-Ichi Hiramatsu[a], Tsuyoshi Kondo[a]

[a]*Department of Neuropsychiatry, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan*
[b]*Miyazato Hospital, 1763-2 Umusa, Nago-shi, Okinawa 905-0006, Japan*

Received 11 March 2003; received in revised form 6 June 2003; accepted 10 June 2003

Abstract

The link between P50 suppression and psychometric schizotypy was previously reported in non-clinical English-speaking subjects; however, whether a similar relationship exists within a different ethnic sample is unknown. Furthermore, whether such a relationship can also be accounted for by such basic personality characteristics as extraversion or neuroticism has not yet been reported. In the present study, we investigated the correlations of P50 suppression with psychometric schizotypy, and with extraversion or neuroticism among non-clinical Japanese. Subjects were 34 healthy volunteers. The auditory P50 potential was obtained using a paired stimulus paradigm. Psychometric schizotypy was assessed using schizotypal personality questionnaire (SPQ). Extraversion and neuroticism were assessed using Maudsley personality inventory (MPI). P50 suppression correlated not only with total SPQ score, but also with extraversion and with neuroticism. However, the partial correlation analysis revealed a significant partial correlation of P50 suppression with SPQ when controlled for extraversion or neuroticism, and a non-significant partial correlation of P50 suppression with extraversion or neuroticism when controlled for SPQ. When subjects were divided into two subgroups according to the mean SPQ score, the degree of P50 suppression was lower in the high than in the low SPQ scorers. Our results indicate that P50 suppression is one of the neurobiological substrates underlying psychometric schizotypy, and that this relationship cannot be accounted for by measures of extraversion or neuroticism.

Keywords: P50 suppression; Psychometric schizotypy; Extraversion; Neuroticism

1. Introduction

Increased interest in studying psychometric schizotypy led to the finding that the schizotypal traits of non-clinical population resemble the syndrome of schizophrenia in the factorial structure. Three major factors extracted from schizotypal traits of the normal population measured using schizotypal personality questionnaire (SPQ, Raine, 1991) were withdrawn, unreality and active (Gruzelier, 1996; Gruzelier and Doig, 1996), or interpersonal, cognitive-perceptual and disorganized (Raine et al., 1994). A similar three-dimensional model was also confirmed in psychiatric patients without psychotic symptoms (Vollema and Hoijtink, 2000). Studies

*Corresponding author. Tel.: +81-98-895-1157; fax: +81-98-895-1419.
E-mail addresses: k018732@eve.u-ryukyu.ac.jp (J. Wang), oki-wjj@umin.ac.jp (J. Wang).

0167-8760/04/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpsycho.2003.06.001

of schizophrenia symptomatology identified similar factors in schizophrenic patients, namely, one negative and two positive syndromes (Liddle and Morris, 1991; Gur et al., 1991). It is suggested that three factors of schizophrenic symptomatology may reflect an exaggeration of the three analogous factors in the general population (Raine et al., 1994). The remarkable similarity between schizotypy and schizophrenia in the factorial structure might be partly associated with the same neural substrate abnormalities.

P50 suppression, which measures the brain function of sensory gating, might be such a neurobiological candidate. It has long been hypothesized that subjects with schizophrenia spectrum disorders have deficits in inhibitory functioning that lead to difficulty in filtering trivial internal and external stimuli, perhaps accounting for the observed attention and cognitive abnormalities. Deficiency in the early sensory gating mechanism has been consistently demonstrated in schizophrenic patients (for instance, Adler et al., 1982; Clementz et al., 1998a; etc) and their first-degree relatives (Clementz et al., 1998b; Siegel et al., 1984; Waldo et al., 1991; Myles-Worsley, 2002). The P50 suppression deficiency was also revealed in schizotypal personality disorder (SPD) subjects as compared with unaffected controls (Cadenhead et al., 2000), suggesting an association of P50 suppression deficits with schizotypal traits as well as with familial genetic vulnerability to schizophrenia. The P50 suppression dysfunction was further related to psychometric schizotypy in a non-clinical population by Croft et al. (2001), with the finding of a lesser degree of P50 suppression in subjects reporting more perceptual anomalies and magical ideation (an unreality factor). However, whether the relationship between P50 suppression and psychometric schizotypy exists within a different ethnic population remains unknown. It is possible that personality traits might be ethnically biased (Reynolds et al., 2000; Chen et al., 1997). The purpose of the present study was to replicate the study by Croft et al. (2001) in a Japanese population. In addition, P50 suppression tests were also administered to one group of schizophrenic patients in the present study, in order to investigate whether the high scorers on schizotypal questionnaires are similar to schizophrenics in terms of P50 suppression (Croft et al., 2001).

While the study of schizotypy in the normal population can be a useful strategy for elucidating the schizophrenic process, controversy over the nature of schizotypy remains. There are two broadly different perspectives on schizotypy: a psychiatric perspective, where schizotypy represents attenuated psychotic symptoms, and a psychological perspective, where schizotypy represents deviant personality traits (Gruzelier, 2003). From the psychological perspective, it is hypothesized that all functional psychoses share an underlying latent trait (psychoticism) which extends beyond psychotic states and can be measured in normal population (Eysenck and Barrett, 1993). Different varieties of psychosis and schizotypy are believed to be manifestations of the other two major personality constructs (extraversion and neuroticism) (Eysenck, 1992). Given that the close relationship between schizotypy and basic personality traits has been reported extensively (for instance, Braunstein-Bercovitz et al., 2002; Ross et al., 2002), it would be more advantageous to investigate whether P50 suppression correlate with such basic personality traits as extraversion or neuroticism simultaneously. This approach might help to clarify whether the link between P50 suppression and psychometric schizotypy is specific, or just an effect secondary to the correlation of P50 suppression with more basic personality traits such as extraversion or neuroticism.

2. Methods

2.1. Subjects

Subjects were 34 paid healthy volunteers, all of whom had provided written informed consent after receiving an explanation of this study. They were recruited from the staff and students of University of the Ryukyus, Okinawa, Japan. The demographic characteristics of the subjects is shown in Table 1. Subjects reported no personal history of neurologic or psychiatric disorders and no familial history of psychiatric illness. There were six light smokers, who smoked approximately 10 cigarettes per day. All subjects were right-handed (Edinburgh hand-

Table 1
The demographic characteristics and means of P50 measurements

	Non-clinical subjects			Schizophrenic patients
	Total subjects	Low SPQ	High SPQ	
Cases	34	21	13	18
Gender (m/f)	16/18	8/13	8/5	13/5
Age	33.4±8.2	32.9±8.5	35.2±7.8	35.7±7.8
S1 latency (ms)	52.2±10.0	51.8±9.7	53.0±10.8	52.7±10.8
S2 latency (ms)	49.5±10.5	50.2±10.6	48.2±10.5	53.6±11.5
S1 amplitude (μV)	1.72±0.78	1.90±0.89	1.42±0.44	1.84±0.91
S2 amplitude (μV)	0.48±0.52	0.31±0.40	0.76±0.59	1.31±1.07
P50 suppression				
S2/S1 ratio	0.30±0.34	0.15±0.22	0.54±0.36	0.87±0.67
S1–S2 difference (μV)	1.24±0.86	1.59±0.81	0.67±0.62	0.54±1.45

edness inventory, Oldfield, 1971). Schizotypal traits were assessed using the Japanese translation of the 74 item Schizotypal personality questionnaire (SPQ) (Raine, 1991; translated by Fujiwara, 1993). The questionnaire was administered to 258 college students (167 males and 91 females) in Shiga University of Medical Science, Shiga, Japan. Compared with the study by Raine (1991), the results showed a lower mean SPQ score of 8.14±7.77 but a similar internal reliability (α= 0.90) and a similar criterion validity (r=0.61, N= 31, P<0.001) (Someya et al., 1994). Basic features of personality were assessed using the Japanese version of Jensen's 80 item Maudsley personality inventory (MPI) (Jensen, 1958; Iwawaki, 1969), which mainly includes two-dimensions: extraversion (24 items) and neuroticism (24 items). MPI was administered to subjects at an interval of 5.9±3.5 months later; however, data for five of the subjects were unobtainable.

Schizophrenic patients were recruited from Miyazato Hospital, Okinawa, Japan, in the present study. Eighteen medicated patients who met DSM-IV criteria for schizophrenia were included. The antipsychotic medication was administered at an equivalent chlorpromazine dosage of 657±266 mg/day. Clinical symptoms were evaluated using the positive and negative syndrome scale (PANSS) (Kay et al., 1987; general mean=28.6, S.D.= 10.6; positive scale mean=13.8, S.D.=6.7; negative scale mean=15.7, S.D.=6.1). There was no group difference in either age ($F_{1,50}$=0.66, P> 0.05) or gender composition (χ^2=3.02, P=0.073) between schizophrenic patients and the non-clinical subjects.

2.2. Procedures

The recording procedures used in the Department of Anatomy, University of Arkansas for Medical Science, USA, were followed (Skinner et al., 1999). Subjects were seated on an armchair in a well-lit, sound-attenuating shielded room. Gold-plated surface electrodes were used, and electrode resistance was maintained at <5 KΩ. The P50 potential was recorded at the vertex (Cz) referred to a frontal electrode (Fz). Eye movements (EOG) were detected by using diagonally placed canthal electrodes, while jaw movements (electromyogram, EMG) were detected by using a lead over the mentalis muscle referred to the chin. A subclavicular ground was used instead of mastoid or earlobe since the subjects wore headphones during the recording. Prior to the recording, the hearing threshold was tested for each subject. The test stimulus was a rarefield click of 0.1 ms duration set at least 50 dB above threshold, usually 95–105 dB, as required. Paired clicks of S1–S2 at an interstimulus interval of 250 ms were delivered once every 5 s to the subjects. This interstimulus interval of the paired clicks was selected in the present study, because several previous reports suggested that a shorter ISI (250 ms) is more sensitive to differences in sensory gating of the

P50 potential (Rasco et al., 2000; Skinner et al., 2002). Signals were collected at 1 sample/ms, recorded and processed using a Synax 2100 (NEC Corporation, Tokyo, 1999). The signal was filtered at 2 Hz–1 kHz for the EEG channel, at 2 Hz–1 kHz for the EOG channel and at 20 Hz–1 kHz for the EMG channel. EEG signals that contained interference from EOG or EMG were excluded from the average. The recording terminated when 64 acceptable trials were averaged. If >8 trials (12.5%) were excluded because of this criterion in order to obtain 64 trials for the average, the subjects were removed from the study. Two subjects were removed from the study for failing to meet this criterion. The subjects were instructed to keep their eyes open and to count the number of trials presented as a means of maintaining vigilance. Only subjects who reported >90% accuracy in stimulus counts were included in the present study. None of the subjects were excluded because of this criterion.

2.3. Data analysis

The P50 potential was identified as the largest positive amplitude wave occurring between 30 and 80 ms after the stimulus. P50 amplitude measures were performed by the classic peak-to-peak method, measuring the amplitude from the preceding negativity (Nb), or from the preceding baseline if Nb was absent. P50 suppression was determined using the ratio of P50 amplitude in response to the second stimulus (S2) divided by the P50 amplitude in response to the first stimulus (S1). It was also assessed using the difference between P50 amplitudes in response to S1 and S2. In order to prevent outliers from disproportionately affecting the group means, the S2/S1 ratios greater than two were assigned the value 2, according to the methods of Nagamoto et al. (1991).

The correlations of P50 measurements with total SPQ score and its three factor scores were computed using Spearman correlating analysis (Bonferroni adjustment used for three factor scores; $\alpha' = 0.0167$). Spearman's measure of association was also used to test for relations between P50 and two MPI factors (Bonferroni adjustment used; $\alpha' = 0.025$). Exploratory partial correlating analyses were performed on P50 suppression with SPQ when adjusting for the effect of extraversion or neuroticism, and also on P50 suppression with extraversion or neuroticism when adjusting for the effect of SPQ. Before the partial correlating analyses, the ratio of S2/S1 amplitude was transformed into its square root to minimize the skewed distribution. No transformation was done with the difference between S1 and S2 amplitudes because of its normal distribution. Pearson correlation analysis was also performed between S1–S2 difference and psychometric measures, and between SPQ and MPI measures.

The non-clinical subjects were further divided into two subgroups of high and low SPQ scorers, according to the mean SPQ score of all subjects. There were 13 subjects with an SPQ score above the mean, and 21 subjects with an SPQ score below the mean. The two subgroups did not differ in age ($F_{1,32} = 0.60$, $P = 0.444$) and the gender composition ($\chi^2 = 1.77$, $P = 0.291$). Subgroup differences in P50 measurements were assessed using Mann–Whitney test. The group differences in P50 variables between the non-clinical subjects and schizophrenic patients were also evaluated using Mann–Whitney test.

3. Results

3.1. Psychometric assessments

The mean total SPQ score of all subjects was 10.5 ± 7.7. The mean SPQ factor score was 5.9 ± 5.4 for the withdrawn, 3.0 ± 3.1 for the unreality and 2.3 ± 2.1 for the active. MPI assessments revealed a mean score of 28.1 ± 11.7 for the extraversion dimension, and 12.0 ± 8.4 for the neuroticism dimension. The intercorrelations between SPQ and MPI are shown in Table 2. The extraversion dimension had a correlation with the total SPQ score ($r = -0.494$, $P = 0.007$) and with the withdrawn factor ($r = -0.694$, $P < 0.001$).

3.2. Correlations of between P50 measurements and psychometric assessments

Spearman's correlations between P50 measurements and psychometric assessments are shown in

Table 2
Pearson's correlation coefficients amongst psychometric variables

		SPQ (n=34)				MPI (n=29)	
		Total	Withdrawn	Unreality	Active	Extraversion	Neuroticism
SPQ							
Total	r	1.0	0.878	0.703	0.754	−0.494	0.380
	P	–	<0.001	<0.001	<0.001	0.007	0.042
Withdrawn	r		1.0	0.360	0.466	−0.694	0.238
	P		–	0.036	0.006	<0.001	0.214
Unreality	r			1.0	0.563	0.067	0.375
	P			–	0.001	0.730	0.045
Active	r				1.0	−0.193	0.375
	P				–	0.316	0.045
MPI							
Extraversion	r					1.0	−0.172
	P					–	0.374
Neuroticism	r						1.0
	P						–

Table 3. The P50 amplitude in response to S1 did not show any correlation with whether total SPQ score or its factor scores. The P50 amplitude in response to S2 correlated with the total SPQ score (r=0.405, P=0.017), although it showed no significant correlation with any factor score of SPQ. The ratio of S2/S1 amplitude demonstrated a very significant correlation not only with the total SPQ score (r=0.525, P=0.001) but also with withdrawn factor (r=0.486, P=0.004). The correlation between the S1–S2 amplitude difference and the total SPQ score was also significant (r=−0.371, P=0.031). In addition, Pearson correlation analysis revealed that the S1–S2 amplitude difference correlated significantly with total SPQ score (r=−0.403, P=0.018; Fig. 1) and with the withdrawn factor (r=−0.442, P=0.009).

Regarding the relationship between P50 measurements and the two MPI subscales, the ratio of S2/S1 amplitude showed a correlation with both extraversion subscale (r=−0.436, P=0.018) and neuroticism subscale (r=0.463, P=0.011). However, when controlling for the effect of the total SPQ score, the square root of the S2/S1 ratio did

Table 3
Spearman's correlation coefficients (and P-values) between P50 and psychometric variables

		SPQ (n=34)				MPI (n=29)	
		Total	Withdrawn	Unreality	Active	Extraversion	Neuroticism
S1 latency	r	0.142	0.061	0.107	0.284	−0.007	−0.090
	P	0.425	0.734	0.548	0.104	0.972	0.644
S2 latency	r	−0.151	−0.120	−0.143	−0.054	−0.001	−0.004
	P	0.394	0.500	0.420	0.761	0.997	0.985
S1 amplitude	r	−0.153	−0.103	−0.119	−0.095	0.060	−0.146
	P	0.388	0.562	0.503	0.592	0.757	0.451
S2 amplitude	r	0.405	0.391	0.202	0.199	−0.365	0.402
	P	0.017	0.022	0.252	0.259	0.051	0.030
S2/S1 ratio	r	0.525	0.486	0.299	0.321	−0.436	0.463
	P	0.001	0.004	0.086	0.064	0.018	0.011
S1–S2 difference	r	−0.371	−0.320	−0.245	−0.220	0.280	−0.345
	P	0.031	0.065	0.163	0.210	0.141	0.067

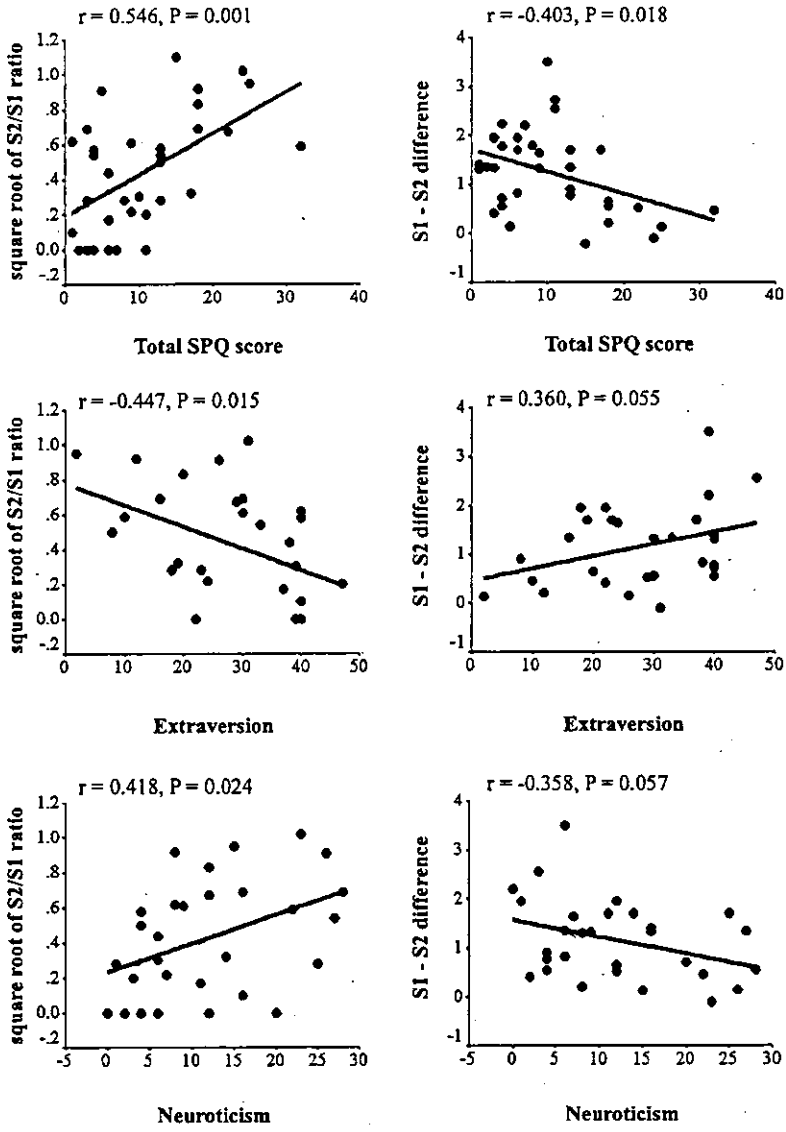

Fig. 1. The linear correlations of P50 suppression with total SPQ score, and with extraversion or neuroticism. P50 suppression was assessed using the ratio of S2/S1 amplitude and the difference between S1 and S2 amplitudes. The ratio was transformed to its square root to minimize its skewed distribution. Both P50 suppression measures demonstrated a significant correlation with total SPQ score. Note the non-significant correlation of S1–S2 difference with extraversion or neuroticism.

not correlate significantly either with extraversion ($r=-0.216$, d.f.$=26$, $P=0.270$) or with neuroticism ($r=0.256$, d.f.$=26$, $P=0.188$). On the contrary, the square root of the S2/S1 ratio still showed a significant partial correlation with SPQ when controlling for extraversion ($r=0.492$, d.f.$=26$, $P=0.008$) or for neuroticism ($r=0.529$, d.f.$=26$, $P=0.004$). Furthermore, this partial correlation between P50 suppression and the total SPQ score was still significant when controlling for both extraversion and neuroticism simultaneously ($r=0.414$, d.f.$=25$, $P=0.032$). Additionally, the S1–

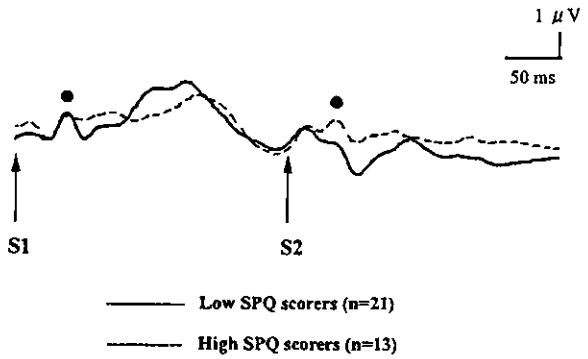

Fig. 2. The grand average waveforms of P50 auditory evoked potentials averaged separately for two non-clinical subgroups. S1: first click; S2: second click. Solid line: low SPQ scorers ($n=21$); dotted line: high SPQ scorers ($n=13$). Note: the P50 potentials in response to S1, indicated by the black dot, were similar between two subgroups; however, the P50 potentials in response to S2, also indicated by the black dot, were inhibited remarkably in the low SPQ scorers but not in the high SPQ scorers.

S2 amplitude difference did not show a significant linear correlation with either extraversion ($r=0.360$, $P=0.055$) or neuroticism ($r=-0.358$, $P=0.057$) (Fig. 1). No correlation of age with any P50 measurement was detected.

3.3. Low SPQ vs. high SPQ scorers

The mean values of P50 variables are shown for all subjects in Table 1. The P50 evoked potentials averaged separately for the low and high SPQ scorers are shown in Fig. 2. Visual inspection found that, while both subgroups had similar P50 responses to the first stimulus (S1), they demonstrated different responses to the second stimulus (S2). Mann–Whitney test revealed that, between the two non-clinical subgroups, there was no difference in P50 latency (S1 latency: $Z=0.25$, $P=0.807$; S2 latency: $Z=1.15$, $P=0.261$) and P50 amplitude in response to S1 ($Z=1.79$, $P=0.076$). A very significant difference was detected with P50 amplitude in response to S2 ($Z=2.79$, $P=0.005$), the ratio of S2/S1 amplitude ($Z=3.40$, $P<0.001$) and the S1–S2 amplitude difference ($Z=3.10$, $P=0.001$), demonstrating a poorer sensory gating function in the high SPQ scorers than in their low SPQ counterparts. Mann–Whitney test was also performed on P50 measurements between male and female subjects, and failed to detect any significant differences (S1 latency: $Z=0.50$, $P=0.621$; S2 latency: $Z=0.59$, $P=0.574$; S1 amplitude: $Z=0.47$, $P=0.646$; S2 amplitude: $Z=1.39$, $P=0.175$; S2/S1 amplitude ratio: $Z=1.35$, $P=0.187$; S1–S2 difference: $Z=0.52$, $P=0.621$).

3.4. Non-clinical subjects vs. schizophrenia patients

Mean values of P50 measurements of 18 schizophrenic patients are also shown in Table 1. Schizophrenic patients demonstrated a poorer P50 suppression as compared to the non-clinical subjects in general (S2/S1 ratio: $Z=3.27$, $P=0.001$). However, separate comparisons of two non-clinical subgroups with schizophrenic patients revealed a significant difference in P50 suppression only between low SPQ scorers and schizophrenic patients (S2/S1 ratio: $Z=3.98$, $P<0.001$), but not between high SPQ scorers and schizophrenic patients (S2/S1 ratio: $Z=1.16$, $P=0.258$) (Fig. 3).

4. Discussion

Raine's SPQ has been applied in our department to screen college students at risk for SPD (Mannan

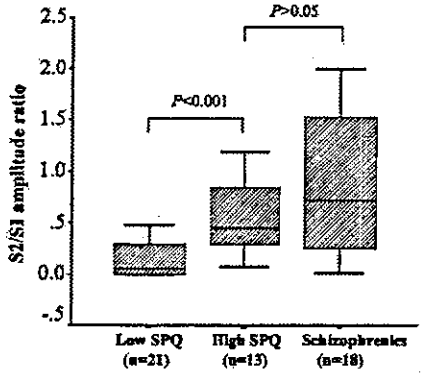

Fig. 3. P50 suppression comparison between non-clinical subjects and schizophrenic patients. Note the non-significant difference in P50 suppression between the high SPQ scorers and the schizophrenics.

et al., 2001). More than 50% of subjects with SPQ scores >20 were later verified as SPD using the structure clinical interview for DSM-III-R. This cut-off point was far below that used by Raine (1991), and was even below the mean SPQ score of their college students. The mean total SPQ score was only 11 in the present study, which together with other SPQ data from Japanese college students (Someya et al., 1994), suggests that Japanese subjects possibly understate their schizotypal traits. However, this kind of cultural difference did not obscure the relationship between psychometric schizotypy and P50 suppression, since the findings of the present study are basically consistent with those of Croft et al. (2001).

In the present study, P50 suppression assessed using the ratio of S2/S1 amplitude or the difference between S1 and S2 amplitudes showed a significant correlation with the total SPQ scores among the non-clinical Japanese. In addition, P50 suppression also correlated with the withdrawn factor score in the present study. In the study of Croft et al. (2001), although an association between P50 suppression and the unreality factor of their PSQ (personality syndrome questionnaire) was emphasized, the correlation between P50 suppression and the subjects' total PSQ score was also significant ($r=0.433$, $P=0.009$; Croft, personal communication). The PSQ is an 80 item questionnaire, which was developed from the SPQ and emphasizes the 3 factorial structure (active, withdrawn and unreality) of schizotypy (Gruzelier, 1996; Gruzelier and Doig, 1996; Croft et al., 2001). Therefore, a main conclusion, which could be drawn from both studies, is that P50 suppression very possibly correlates with schizotypy as a whole syndrome. This could also be supported by the tendency towards a relationship between P50 suppression and the active subscale in both studies. The role of thalamo-cortical activation systems is the key to understanding schizotypy and schizophrenia. It is proposed that lateral shifts in the orchestration of hemispheric activation via the thalamocortical activation systems underpins the activated and withdrawn syndromes, which are shifted to the left with activated syndrome and shifted to the right with withdrawn syndrome (Gruzelier, 2002, 2003). A generalized dysfunction between the non-specific arousal system and specific thalamocortical input is possibly associated with the 'unreality' experience (Gruzelier, 2002, 2003).

P50 suppression possibly measures the GABA mediated inhibitory processes in the thalamo-limbic-cortical circuitry. Freedman and his colleagues identified the pyramid neurons of the CA3 region of the hippocampus as the sources of cerebral-evoked P50 response, and reported that the P50 suppression deficit in schizophrenic patients was related to desensitization of the alpha-7 nicotinc receptor on the hippocampal GABA-ergic interneurons (Adler et al., 1992; Griffith et al., 1998; Freedman et al., 2002). There is also strong evidence suggesting that the P50 potential has neural origin in the temporal auditory cortex, and in the reticular activating system (RAS) of the brainstem, especially the ascending projection of PPN (pedunculopontine nucleus) (Reite et al., 1988; Makela et al., 1994; Reese et al., 1995; Garcia-Rill et al., 1995). Our work on animal equivalents of P50 potential (P13 in rat) lends further support to the probable brainstem source of this waveform (Miyazato et al., 1999a,b). However, the P50 potential, as well as other mid-latency EP components, may be produced by underlying stimulus-bound synchronous oscillation in the gamma frequency band (40 Hz); and P50 suppression may be a proxy for gamma band response (GBR) suppression (Basar et al., 1987; Clementz et al., 1997; Muller et al., 2001; Crawford et al., 2002). The neural mechanisms of synchronous gamma activity mainly include the GABA-ergic interneuron network model and the thalamo-cortical arousal model (Lee et al., 2003).

In the present study, the non-clinical subjects with high SPQ scores not only had a poorer P50 suppression than their low SPQ counterparts, but also demonstrated the deficiency similar in severity to that of schizophrenic patients, which further attests to the continuum of subclinical schizotypy-clinical schizophrenia. In the study of Croft et al. (2001), the P50 suppression results of low and high 'unreality' subjects were also suggested to be consistent with comparisons of controls with either schizophrenia patients or SPD subjects in the literature. Additionally, subjects who met predeter-

mined criteria for 'schizotaxia' (a concept similar to negative schizotypy) showed a positive response to low doses of risperidone (Tsuang et al., 2002). All these findings are in line with the psychiatric perspective on schizotypy where schizotypy represents attenuated psychotic symptoms (Gruzelier, 2003).

The present study further revealed that the relationship between P50 suppression and schizotypy in the non-clinical subjects may be uncontaminated by basic personality features of extraversion or neuroticism as measured using MPI, suggesting that a specific relationship exists between this neurocognitive function and schizotypy. This is consistent with the findings reported in the families of schizophrenics (Freedman et al., 2002). In these families, most of the psychopathology other than schizophrenia itself is found in siblings who do not have P50 inhibitory deficits, whereas individuals with P50 inhibitory deficits have some symptoms of schizotypal personality disorder. The specific relationship between schizotypy and P50 suppression revealed in the present study might confound the psychological perspective on schizotypy suggested by Eysenck (1992). However, we acknowledge that, for several reasons, it is difficult practically to disentangle the effect of basic personality characteristics on the relationship between schizotypy and sensory gating function. First, the close intercorrelations among schizotypy, extraversion, neuroticism and other personality characteristics were consistently reported (for instance, Ross et al., 2002; etc). The correlation between negative schizotypy and extraversion was also very significant in the present study. Second, findings to the contrary were reported. In the high schizotypals, the anxiety component of schizotypy, more than the perceptual-disorganization (schizophrenia-like) component, was found to account for the neurocognitive deficit of latent inhibition (LI), which is used to assess selective attention processes and distraction by irrelevant stimuli (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz et al., 2002). The P50 suppression deficits also occurred in the high-anxious individuals such as posttraumatic stress disorder patients (Gillette et al., 1997; Neylan et al., 1999; Skinner et al., 1999). Whether the relationship between P50 suppression and schizotypy could be accounted for by the anxiety component needs to be clarified in future studies. Third, the neurobiological basis of personality overlaps with the mechanism of P50 suppression. For instance, extraversion has been interpreted as the autoregulative behavioral response to a low tonic activity of the non-specific activating system (Eysenck, 1967; Hegerl et al., 1995).

The main limitation of the present study was the small sample size, especially in relation to psychometric assessments. This limitation possibly contributed to the finding that the correlations between P50 suppression and the unreality or the active factors of schizotypy were not significant. Confirmation of the findings in additional subjects is necessary. In conclusion, the present study suggests that P50 suppression is one of the neurobiological substrates underlying psychometric schizotypy, and that this relationship cannot be accounted for by basic personality measures of extraversion or neuroticism.

Acknowledgments

This study was supported by Japan Grant-in-Aid for Scientific Research (No. 13671022).

References

Adler, L.E., Pachtman, E., Franks, R.D., Pecevich, M., Waldo, M.C., Freedman, R., 1982. Neurophysiological evidence for a defect in neuronal mechanisms involved in sensory gating in schizophrenia. Biol. Psychiatry 17, 639–654.

Adler, L.E., Hoffer, L.J., Griffith, J., Waldo, M.C., Freedman, R., 1992. Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. Biol. Psychiatry 32, 607–616.

Basar, E., Rosen, B., Basar-Eroglu, C., Greitschus, F., 1987. The association between 40-Hz EEG and the middle latency response of the auditory evoked potential. Int. J. Neurosci. 33, 103–117.

Braunstein-Bercovitz, H., 2000. Is the attentional dysfunction in schizotypy related to anxiety? Schizophr. Res. 46, 255–267.

Braunstein-Bercovitz, H., Rammsayer, T., Gibbons, H., Lubow, R.E., 2002. Latent inhibition deficits in high-schizotypal normals: symptom-specific or anxiety-related? Schizophr. Res. 53, 109–121.

Cadenhead, K.S., Light, G.A., Geyer, M.A., Braff, D.L., 2000. Sensory gating deficits assessed by the P50 event-related potential in subjects with schizotypal personality disorder. Am. J. Psychiatry 157, 55–59.

Chen, W.J., Hsiao, C.K., Lin, C.C., 1997. Schizotypy in community samples: the three-factor structure and correlation with sustained attention. J. Abnorm. Psychol. 106, 649–654.

Clementz, B.A., Blumenfeld, L.D., Cobb, S., 1997. The gamma band response may account for poor P50 suppression in schizophrenia. Neuroreport 8, 3889–3893.

Clementz, B.A., Geyer, M.A., Braff, D.L., 1998a. Multiple site evaluation of P50 suppression among schizophrenia and normal comparison subjects. Schizophr. Res. 30, 71–80.

Clementz, B.A., Geyer, M.A., Braff, D.L., 1998b. Poor P50 suppression among schizophrenia patients and their first-degree biological relatives. Am. J. Psychiatry 155, 1691–1694.

Crawford, H.J., McClain-Furmanski, D., Castagnoli, N., Castagnoli, K., 2002. Enhancement of auditory sensory gating and stimulus-bound gamma band (40 Hz) oscillations in heavy tobacco smokers. Neurosci. Lett. 317, 151–155.

Croft, R.J., Lee, A., Bertolot, J., Gruzelier, J.H., 2001. Associations of P50 suppression and desensitization with perceptual and cognitive features of 'unreality' in schizotypy. Biol. Psychiatry 50, 441–446.

Eysenck, H.J., 1967. The biological basis of personality. Springfield, III: Thomas C.

Eysenck, H.J., 1992. The definition and measurement of psychoticism. Pers. Individ. Differ. 13, 757–785.

Eysenck, H.J., Barrett, P., 1993. The nature of schizotypy. Psychol. Rep. 73, 59–63.

Freedman, R., Adler, L.E., Olincy, A., Waldo, M.C., Ross, R.G., Stevens, K.E., et al., 2002. Input dysfunction, schizotypy, and genetic models of schizophrenia. Schizophr. Res. 54, 25–32.

Fujiwara, T., 1993. Japanese translation of 'The SPQ: a scale for the assessment of schiztypal personality based on DSM-III-R criteria'. Adv. Schizophr. Res. 3, 21–25 Japanese.

Garcia-Rill, E., Biedermann, J.A., Chambers, T., Skinner, R.D., Mrak, R.E., Husain, M., et al., 1995. Mesopontine neurons in schizophrenia. Neuroscience 66, 321–335.

Gillette, G.M., Skinner, R.D., Rasco, L.M., Fielstein, E.M., Davis, D.H., Pawelak, J.E., et al., 1997. Combat veterans with posttraumatic stress disorder exhibit decreased habituation of the P1 midlatency auditory evoked potential. Life Sci. 61, 1421–1434.

Griffith, J.M., O'Neill, J.E., Petty, F., Garver, D., Young, D., Freedman, R., 1998. Nicotinic receptor desensitization and sensory gating deficits in schizophrenia. Biol. Psychiatry 44, 98–106.

Gruzelier, J.H., 1996. The factorial structure of schizotypy: part I. Affinities with syndromes of schizophrenia. Schizophr. Bull. 22, 611–620.

Gruzelier, J.H., 2002. A Janusian perspective on the nature, development and structure of schizophrenia and schizotypy. Schizophr. Res. 54, 95–103.

Gruzelier, J.H., 2003. Theory, methods and new directions in the psychophysiology of the schizophrenic process and schizotypy. Int. J. Psychophysiol. 48, 221–245.

Gruzelier, J.H., Doig, A., 1996. The factorial structure of schizotypy: part II. Cognitive asymmetry, arousal, handedness, and sex. Schizophr. Bull. 22, 621–634.

Gur, R.E., Mozley, P.D., Resnick, S.M., Levick, S., Erwin, R., Saykin, A.J., et al., 1991. Relations among clinical scales in schizophrenia. Am. J. Psychiatry 148, 472–478.

Hegerl, U., Gallinat, J., Mrowinski, D., 1995. Sensory cortical processing and the biological basis of personality. Biol. Psychiatry 37, 467–472.

Iwawaki, M., 1969. Sin Seikaku Kensa Hou—Maudsley Personality Inventory. Seisin Syobo, Tokyo, pp. 115–177 Japanese.

Jensen, A.R., 1958. The Maudsley personality inventory. Acta Psychol. 14, 314–325.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.

Lee, K.H., Williams, L.M., Breakspear, M., Gordon, E., 2003. Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. Brain Res. Rev. 41, 57–78.

Liddle, P.F., Morris, D.L., 1991. Schizophrenic syndromes and frontal lobe performance. Br. J. Psychiatry 158, 340–345.

Makela, J.P., Hamalainen, M., Hari, R., McEvoy, L., 1994. Whole-head mapping of middle-latency auditory evoked magnetic fields. Electroencephalogr. Clin. Neurophysiol. 92, 414–421.

Mannan, M.R., Hiramatsu, K.I., Hokama, H., Ohta, H., 2001. Abnormalities of auditory event-related potentials in students with schizotypal personality disorder. Psychiatry Clin. Neurosci. 55, 451–457.

Miyazato, H., Skinner, R.D., Cobb, M., Andersen, B., Garcia-Rill, E., 1999a. Midlatency auditory-evoked potentials in the rat: effects of interventions that modulate arousal. Brain Res. Bull. 48, 545–553.

Miyazato, H., Skinner, R.D., Garcia-Rill, E., 1999b. Sensory gating of the P13 midlatency auditory evoked potential and the startle response in the rat. Brain Res. 822, 60–71.

Muller, M.M., Keil, A., Kissler, J., Gruber, T., 2001. Suppression of the auditory middle-latency response and evoked gamma-band response in a paired-click paradigm. Exp. Brain Res. 136, 474–479.

Myles-Worsley, M., 2002. P50 sensory gating in multiplex schizophrenia families from a Pacific island isolate. Am. J. Psychiatry 159, 2007–2012.

Nagamoto, H.T., Adler, L.E., Waldo, M.C., Griffith, J.M., Freedman, R., 1991. Gating of auditory response in schizophrenics and normal controls. Effects of recording site and stimulation interval on the P50 wave. Schizophr. Res. 4, 31–40.

Neylan, T.C., Fletcher, D.J., Lenoci, M., McCallin, K., Weiss, D.S., Schoenfeld, F.B., et al., 1999. Sensory gating in chronic posttraumatic stress disorder: reduced auditory P50 suppression in combat veterans. Biol. Psychiatry 46, 1656–1664.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Raine, A., 1991. The SPQ: a scale for the assessment of schiztypal personality based on DSM-III-R criteria. Schizophr. Bull. 17, 556–564.

Raine, A., Reynolds, C., Lencz, T., Scerbo, A., Triphon, N., Kim, D., 1994. Cognitive-perceptual, interpersonal, and disorganized features of schizotypal personality. Schizophr. Bull. 20, 191–201.

Rasco, L., Skinner, R.D., Garcia-Rill, E., 2000. Effect of age on sensory gating of the sleep state-dependent P1/P50 midlatency auditory evoked potential. Sleep Res. Online 3, 97–105.

Reese, N.B., Garcia-Rill, E., Skinner, R.D., 1995. The pedunculopontine nucleus–auditory input, arousal and pathophysiology. Prog. Neurobiol. 47, 105–133.

Reite, M., Teale, P., Zimmerman, J., Davis, K., Whalen, J., 1988. Source location of a 50 ms latency auditory evoked field component. Electroencephalogr. Clin. Neurophysiol. 70, 490–498.

Reynolds, C.A., Raine, A., Mellingen, K., Venables, P.H., Mednick, S.A., 2000. Three-factor model of schizotypal personality: invariance across culture, gender, religious affiliation, family adversity, and psychopathology. Schizophr. Bull. 26, 603–618.

Ross, S.R., Lutz, C.J., Bailley, S.E., 2002. Positive and negative symptoms of schizotypy and the five-factor model: a domain and facet level analysis. J. Pers. Assess. 79, 53–72.

Siegel, C., Waldo, M.C., Mizner, G., Adler, L.E., Freedman, R., 1984. Deficits in sensory gating in schizophrenia patients and their relatives. Arch. Gen. Psychiatry 41, 607–612.

Skinner, R.D., Rasco, L.M., Fitzgerald, J., Karson, C.N., Matthew, M., Williams, D.K., et al., 1999. Reduced sensory gating of the P1 potential in rape victims and combat veterans with posttraumatic stress disorder. Depress. Anxiety 9, 122–130.

Skinner, R.D., Miyazato, H., Garcia-Rill, E., 2002. The sleep-state dependent P50 auditory evoked potential in neuropsychiatric diseases. In: Hirata, K., Koga, Y., Nagata, K., Yamazaki, K. (Eds.), Recent Advances in Human Brain Mapping. Elsevier, Amsterdam, pp. 813–825.

Someya, T., Sasaki, T., Orita, S., Takahashi, S., 1994. About reliability and validity of schizotypal personality questionnaire (SPQ). Arch. Psychiatr. Clin. Eval. 5, 98 Japanese.

Tsuang, M.T., Stone, W.S., Tarbox, S.I., Faraone, S.V., 1 Mar 2002. An integration of schizophrenia with schizotypy: identification of schizotaxia and implications for research on treatment and prevention. Schizophr. Res. 54 (1–2), 169–175.

Vollema, M.G., Hoijtink, H., 2000. The multidimensionality of self-report schizotypy in a psychiatric population: an analysis using multidimensional Rasch models. Schizophr. Bull. 26, 565–575.

Waldo, M.C., Carey, G., Myles-Worsley, M., Cawthra, E., Adler, L.E., Nagamoto, H.T., et al., 1991. Codistribution of a sensory gating deficit and schizophrenia in multi-affected families. Psychiatry Res. 39, 257–268.

No association of *EGF* polymorphism with schizophrenia in a Japanese population

Yuichiro Watanabe, Naoki Fukui, Tatsuyuki Muratake,[CA] Naoshi Kaneko and Toshiyuki Someya

Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Asahimachi-dori 1-757, Niigata 951-8510, Japan

[CA]Corresponding Author: mura@med.niigata-u.ac.jp

Received 23 December 2004; accepted 11 January 2005

Epidermal growth factor (EGF) signal regulates the development of dopaminergic neurons and monoamine metabolism. It is suggested that EGF protein levels are decreased in the brain and blood of patients with schizophrenia. A recent study has reported that a polymorphism in *EGF* gene (rs4444903) is associated with schizophrenia in Finnish men. To confirm this association for another population in larger samples, we conducted a case–control association study on a Japanese population (337 cases and 421 controls). No significant difference was observed in both the allelic and genotype distribution between cases and controls in women, men and total samples. Our results suggest that the polymorphism in *EGF* gene might not confer increased susceptibility for schizophrenia in a Japanese population. *NeuroReport* 16:403–405 © 2005 Lippincott Williams & Wilkins.

Key words: Association study; Cytokine; Epidermal growth factor; Schizophrenia; Single nucleotide polymorphism

INTRODUCTION

Schizophrenia is a complex genetic disorder that affects approximately 1% of the population worldwide. Cytokines might be implicated in the etiology or pathology of schizophrenia [1], although the pathogenesis of schizophrenia is still unclear. Epidermal growth factor (EGF) has several effects on dopaminergic neurons: stimulating neurite outgrowth, increasing dopamine uptake and enhancing long-term survival in cultured dopaminergic neurons [2,3]. Subchronic peripheral EGF administration into neonatal rats resulted in abnormal dopamine metabolism in the striatum and brain stem of adult rats [4]. In parallel, rats treated with EGF during the neonatal period displayed various behavioral abnormalities in adulthood [4,5]. Some of these behavioral abnormalities, which are also present in an animal model of schizophrenia, were ameliorated by subchronic treatment with antipsychotics [4]. In the post-mortem brain and fresh serum of patients with schizophrenia, EGF and its receptor protein levels were altered [6]. Shahbazi *et al.* [7] identified a G to A polymorphism at position 61 in the 5′ untranslated region of *EGF* gene, located on chromosome 4q25–q27. Interestingly, blood mononuclear cell cultures from individuals with G/G or A/G genotypes produced significantly more EGF than cells from individuals with A/A genotype, suggesting that this polymorphism might be functional [7]. Recently, Anttila *et al.* [8] reported that the G allele was associated with schizophrenia in Finnish men. To assess whether this functional polymorphism in *EGF* gene could be implicated in vulnerability to schizophrenia, we performed a case–control association study in Japanese samples.

MATERIALS AND METHODS

Participants: Participants included 337 patients with schizophrenia (158 women and 179 men) and 421 controls (207 women and 214 men). The mean ages and SD of the cases and controls were 42.0±15.0 and 38.3±10.5 years, respectively. All the participants were unrelated Japanese living in Niigata. Patients meeting the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria for schizophrenia were recruited from nine hospitals: Kohdo Hospital, Matsuhama Hospital, Minamihama Hospital, Niigata Prefectural Psychiatric Center, Niigata University Hospital, Ohjima Hospital, Sado General Hospital, Shirone-Kensei Hospital and Shirone-Midorigaoka Hospital. The controls were healthy volunteers with no history of psychiatric disorders. Written informed consent was obtained from all patients before their participation in this study. This study was approved by the Ethics Committee on Genetics of Niigata University School of Medicine.

Genotyping: Genomic DNA was extracted from peripheral blood by the standard phenol/chloroform method. A single nucleotide polymorphism at position 61 of *EGF* gene (rs4444903) was genotyped by the TaqMan 5′-exonuclease assay. The primer and probe set was designed and synthesized by Applied Biosystems (Foster City, California, USA). We carried out polymerase chain reaction amplification using TaqMan 2 × Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5 ng of DNA, 0.9 μM of each primer and 200 nM of each probe in a total volume of 5 μl. Each 96-well plate contained 94 samples and two no-DNA template controls. Thermal cycler conditions were 95°C for

Table 1. Allele and genotype frequencies of *EGF* gene polymorphism in cases and controls.

	Women		Men		Total	
	Cases (n=158)	Controls (n=207)	Cases (n=179)	Controls (n=214)	Cases (n=337)	Controls (n=421)
Allele						
A	96 (30.4%)	117 (28.3%)	117 (32.7%)	138 (32.2%)	213 (31.6%)	255 (30.0%)
G	220 (69.6%)	297 (71.7%)	241 (67.3%)	290 (67.8%)	461 (68.4%)	587 (70.0%)
Genotype						
A/A	10 (6.3%)	18 (8.7%)	19 (10.6%)	22 (10.3%)	29 (8.6%)	40 (9.5%)
A/G	76 (48.1%)	81 (39.1%)	79 (44.1%)	94 (44.0%)	155 (46.0%)	175 (41.5%)
G/G	72 (45.6%)	108 (52.2%)	81 (45.3%)	98 (45.7%)	153 (45.4%)	206 (49.0%)

10 min, 40 cycles of 92°C for 15 s and 60°C for 1 min. Fluorescence and allelic discrimination were measured with an ABI PRISM 7900HT Sequence Detection System using SDS 2.0 software (Applied Biosystems).

Statistical analysis: Deviation of Hardy–Weinberg equilibrium was tested by using the χ^2 test for goodness of fit. The allele and genotype frequencies between cases and controls were compared using the χ^2 test. A probability level of $p<0.05$ was considered to be statistically significant.

RESULTS

The genotype distributions in cases and controls had no deviation from Hardy–Weinberg equilibrium (χ^2=1.4, df=2, p=0.50 for cases; χ^2=0.1, df=2, p=0.95 for controls). Allele and genotype frequencies of *EGF* gene polymorphism (rs4444903) are presented in Table 1. No significant difference was observed in the allelic distribution between cases and controls in women, men and total samples (χ^2=0.3, df=1, p=0.59; χ^2=0.003, df=1, p=0.96 and χ^2=0.2, df=1, p=0.62, respectively). Also, no significant difference was observed in the genotype distribution between cases and controls in women, men and total samples (χ^2=3.1, df=2, p=0.21; χ^2=0.02, df=2, p=0.99 and χ^2=1.5, df=2, p=0.47, respectively).

DISCUSSION

Anttila *et al.* [8] found that the functional polymorphism in *EGF* gene was associated with schizophrenia in Finnish men. In contrast, we failed to confirm this association in our Japanese samples, suggesting that this polymorphism does not play a major role in conferring susceptibility to schizophrenia. This discrepancy might stem from an ethnic heterogeneity in the functional polymorphism in *EGF* gene as in the promoter variants of *tumor necrosis factor* α gene [9] in schizophrenia. Our sample size (337 cases and 421 controls) is larger than that of Anttila *et al.* (94 cases and 98 controls). Using the Genetic Power Calculation [10], our sample has a power of 0.77 to detect a significant association between the G allele and schizophrenia with an α of 0.05, assuming a disease prevalence of 0.01, a risk allele frequency of 0.7, and a genotypic relative risk of 2.0 for G/G and of 1.5 for A/G. Accordingly, the likelihood of type II error with our sample size appears to be considerably low. However, additional studies of the functional polymorphism in *EGF* gene to evaluate across other ethnic populations are needed to draw a conclusion.

Futamura *et al.* [6] reported that EGF protein levels were decreased in the prefrontal cortex and striatum of patients with schizophrenia in Japanese samples. Conversely, EGF receptor expression was significantly elevated in the prefrontal cortex [6]. Serum EGF protein levels were also decreased in patients, even in young drug-free patients [6]. In experimental animals, EGF treatment during the neonatal period transiently increased tyrosine hydroxylase expression and resulted in abnormal dopamine metabolism in adulthood [4]. The neonatally EGF-treated rats displayed schizophrenia-like behavioral impairments such as hyperactivity, decreased prepulse inhibition, decreased social interaction and hypersensitization to psychostimulants in adulthood [4,5]. These observations suggest that EGF signaling might be implicated in the etiology or pathology of schizophrenia. Although we found no association between the functional polymorphism in *EGF* gene and schizophrenia in our Japanese samples, there is the possibility that the genes of other EGF signaling molecules such as EGF family cytokines and their receptors are associated with schizophrenia [11]. In addition, our result also does not imply the exclusion of the contribution of other polymorphisms in *EGF* gene to the pathogenesis of schizophrenia, because *EGF* gene is a very large gene spanning over 110 kbp.

CONCLUSION

We conclude that the functional polymorphism in *EGF* gene (rs4444903) does not play a major role in conferring susceptibility to schizophrenia in a Japanese population. However, several studies indicate that EGF signaling might be implicated in the etiology or pathology of schizophrenia. It will be necessary to evaluate EGF signaling molecules and other polymorphisms in *EGF* gene in several ethnic populations for association with schizophrenia.

Electronic-Database Information

URLs for data presented herein are as follows:

Genetic Power Calculator, http://wbiomed.curtin.edu.au/genepop/

National Center for Biotechnology Information, Single Nucleotide Polymorphism Database, http://www.ncbi.nlm.nih.gov/SNP/ (for reference identification numbers for SNP).

REFERENCES

1. Nawa H, Takahashi M, Patterson PH. Cytokine and growth factor involvement in schizophrenia–support for the developmental model. *Mol Psychiatry* 2000; 5:594–603.
2. Yamada M, Ikeuchi T, Hatanaka H. The neurotrophic action and signaling of epidermal growth factor. *Prog Neurobiol* 1997; 51:19–37.
3. Wong RWC, Guillaud L. The role of epidermal growth factor and its receptors in mammalian CNS. *Cytokine Growth Factors Rev* 2004; 15:147–156.
4. Futamura T, Kakita A, Tohmi M, Sotoyama H, Takahashi H, Nawa H. Neonatal perturbation of neurotrophic signaling results in abnormal sensorimotor gating and social interaction in adults: implication for epidermal growth factor in cognitive development. *Mol Psychiatry* 2003; 8:19–29.
5. Mizuno M, Malta RS Jr, Nagano T, Nawa H. Conditioned place preference and locomotor sensitization after repeated administration of cocaine or methamphetamine in rats treated with epidermal growth factor during the neonatal period. *Ann NY Acad Sci* 2004; 1025:612–618.
6. Futamura T, Toyooka K, Iritani S, Niizato K, Nakamura R, Tsuchiya K *et al.* Abnormal expression of epidermal growth factor and its receptor in the forebrain and serum of schizophrenic patients. *Mol Psychiatry* 2002; 7:673–682.
7. Shahbazi M, Pravica V, Nasreen N, Fakhoury H, Fryer AA, Strange RC *et al.* Association between functional polymorphism in *EGF* gene and malignant melanoma. *Lancet* 2002; 359:397–401.
8. Anttila S, Illi A, Kampman O, Mattila KM, Lehtimäki T, Leinonen E. Association of EGF polymorphism with schizophrenia in Finnish men. *Neuroreport* 2004; 15:1215–1218.
9. Duan S, Xu Y, Chen W, Liu Z, Guo T, Gao J *et al.* No association between the promoter variants of tumor necrosis factor alpha (TNF-α) and schizophrenia in Chinese Han population. *Neurosci Lett* 2004; 366:139–143.
10. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; 19:149–150.
11. Corfas G, Roy K, Buxbaum JD. Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci* 2004; 7:575–580.

Acknowledgements: We thank the patients, their families and the healthy volunteers for their participation. We also thank Hiromi Tanabe and Hiroshi Kusano for excellent technical assistance.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 121–125
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of fluvoxamine and its metabolite fluvoxamino acid by liquid–liquid extraction and column-switching high-performance liquid chromatography

Norio Yasui-Furukori[a,*], Yoshimasa Inoue[b], Sunao Kaneko[a], Koichi Otani[c]

[a] *Department of Neuropsychiatry, Hirosaki University School of Medicine, Zaifu cho 5, Hirosaki 0368562, Japan*
[b] *Pharmaceutical Technology Division, Mitsubishi Pharma, Fukuoka, Japan*
[c] *Department of Neuropsychiaty, Yamagata University School of Medicine, Yamagata, Japan*

Received 20 July 2004; received in revised form 23 September 2004; accepted 25 September 2004
Available online 10 November 2004

Abstract

This study describes a new simultaneous determination of fluvoxamine and fluvoxamino acid by automated column-switching high-performance liquid chromatography. The test compounds were extracted from 1.5 ml of plasma using chloroform–toluene (15:85, v/v), and the extract was injected into a hydrophilic metaacrylate polymer column for clean-up and a C18 analytical column for separation. The mobile phase for separation consisted of phosphate buffer (0.02 M, pH 4.6), acetonitrile and perchloric acid (60%) (62.4:37.5:0.1, v/v/v) and was delivered at a flow rate of 0.6 ml/min. The peak was detected using a UV detector set at 254 nm. The method was validated for the concentration range 0.8–153.6 ng/ml for fluvoxamine and 0.6–115.2 ng/ml for fluvoxamino acid, and their good linearity ($r > 0.998$) were confirmed. Intra-day coefficient variations (CVs) for fluvoxamine and fluvoxamino acid were less than 6.6 and 6.0%, respectively. Inter-day CVs for corresponding compounds were 6.3 and 6.5%, respectively. Relative errors ranged from −18 to 9% and mean recoveries were 96–100%. The limit of quantification was 1.2 and 0.9 ng/ml for fluvoxamine and fluvoxamino acid, respectively. This method shows successful application for pharmacokinetic studies and therapeutic drug monitoring.

Keywords: Fluvoxamine; Fluvoxamino acid; HPLC; Column-switching

1. Introduction

Fluvoxamine, a selective serotonin (5-HT) reuptake inhibitor, shows similar therapeutic efficacy to tricyclic antidepressants, but fewer anticholinergic and cardiovascular side effects [1]. Nausea is the most common side effect of fluvoxamine [1]. Fluvoxamine undergoes extensive metabolism, and the major metabolite in human urine is fluvoxamino acid [2]. A clinical trial showed that the steady-state plasma concentration of fluvoxamino acid was correlated with the therapeutic outcome [3]. It appears that fluvoxamino acid as well as fluvoxamine contributes to the antidepressant effect during fluvoxamine treatment, and the combined steady-state plasma concentration correlates well with the therapeutic outcome. On the other hand, the fluvoxamino acid/fluvoxamine ratio was significantly different between CYP2D6 genotypes [4]. From not only a clinical point of view but also a pharmacokinetic point of view, therefore, monitoring of both fluvoxamine and fluvoxamino acid is clinically relevant.

Several HPLC methods with ultraviolet detection for the determination of fluvoxamine concentration have been widely reported [5–18]. Although simultaneous determination of antidepressants including fluvoxamine has been reported [16], potential active metabolite has not been included in these studies. Of these reports, only a report by Ohkubo et al. [16] described determination of steady-state plasma concentration of fluvoxamine and fluvoxamino acid in de-

* Corresponding author. Tel.: +81 172 39 5066; fax: +81 172 39 5067.
E-mail address: yasufuru@cc.hirosaki-u.ac.jp (N. Yasui-Furukori).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.09.037

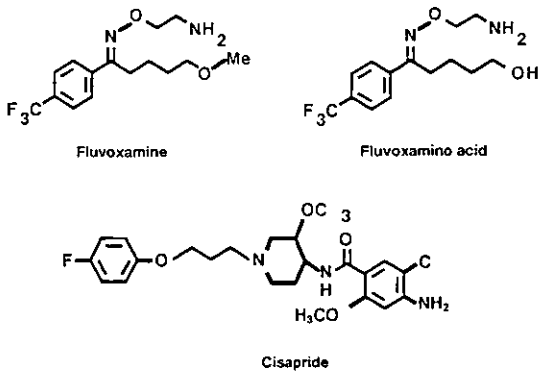

Fig. 1. Chemical structure analogues of fluvoxamine, fluvoxamino acid and cisapride.

pressed patients. However, because preclinical pharmacokinetic data showed C_{max} of 10–15 ng/ml after a single oral dose (50 mg) of fluvoxamine [19], the quantification limits (10 ng/ml) of both compounds in their report [16] are too high to obtain pharmacokinetic parameters of these compounds. In the present study, therefore, we describe an automated column-switching HPLC with ultraviolet detection for determination of fluvoxamine and fluvoxamino acid in plasma using a simple liquid–liquid extraction. The sensitive system allows measurement of plasma concentrations of fluvoxamine and fluvoxamino acid up to 36 and 12 h, respectively, after an oral administration of 50 mg fluvoxamine in pharmacokinetic study.

2. Experimental

2.1. Chemicals

Fluvoxamine and fluvoxamino acid (Fig. 1) were synthesized by Wako Pure Chemical Industries (Osaka, Japan). The structures of these compounds were confirmed using NMR spectra and FAB–MS. The purity of these materials was more than 99.5%. Cisapride as internal standard (IS) (Fig. 1) was kindly provided by Yoshitomi Pharmaceutical (Osaka, Japan). Potassium phosphate monobasic, acetonitrile, perchloric acid, toluene and chloroform were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

Stock solutions of fluvoxamine, fluvoxamino acid and IS for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 76.8, 57.6 and 1000 μg/ml, respectively. High-concentration working standard solutions of fluvoxamine (768 ng/ml), fluvoxamino acid (576 ng/ml) and IS (10 μg/ml) were obtained by 100 times dilution of each stock solution with purified water. Low-concentration working standard solution of fluvoxamine (96 ng/ml) and fluvoxamino acid (72 ng/ml) were obtained by further diluting the high-concentration working standard solution eight times with purified water. Stock solutions were stable at 4 °C for at least 3 months. Drug-free plasma from healthy donors was used for validation studies. Calibration curves were prepared by spiking 12.5, 25, 50 μl of low-concentration working standard solutions and 12.5, 25, 50, 100, 150, 200, 300 μl of high-concentration working standard solutions in 1.5 ml of blank plasma (final volume) to yield the following final concentrations: 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 76.8, 102.4 and 153.6 ng/ml for fluvoxamine and 0.6, 1.2, 2.4, 4.8, 9.6, 19.2, 38.4, 57.6, 76.8 and 115.2 ng/ml for fluvoxamino acid. Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak-height ratio versus the respective concentration of fluvoxamine and fluvoxamino acid. Stock solution of each compound was separately prepared for quality controls in the same manner as for standard curves. Working plasma solutions were obtained by dilution of new stock solutions 1000 times (360 μg/ml for fluvoxamine and 270 μg/ml for fluvoxamino acid) with blank plasma to yield 360 ng/ml for fluvoxamine and 270 ng/ml for fluvoxamino acid. Quality control samples were obtained by spiking 5–400 μl of working plasma solutions in 1.5 ml of blank plasma (final volume) to yield the final concentrations range of 1.2, 24 and 96 ng/ml for fluvoxamine and 0.9, 18 and 72 ng/ml for fluvoxamino acid, and kept at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.3. Sample collections

A tablet containing 50 mg of fluvoxamine (Luvox®, Fujisawa Pharmaceutical, Osaka, Japan) was orally administered to each of 10 healthy volunteers. Their mean (range) age was 31 years (25–36 years) and body weight was 64 kg (54–85 kg). Blood samples were obtained before and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h after the dosing. Blood samples were collected in heparinized tubes and centrifuged immediately at 2500 × *g* for 10 min. The plasma was stored at −20 °C until analysis. The study protocol was approved by Ethical Committee of Hirosaki University School of Medicine and Ethical Committee of Yamagata University School of Medicine and a written informed consent was given from each subject.

2.4. Apparatus

The column-switching HPLC system consisted of two Shimadzu LC-10AT high-pressure pumps (for eluent A and B), a Shimadzu CTO-10A column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan), a Tosoh multiple autovalve PT-8000, and a Tosoh autosampler AS-8020 (500 μL injection volume) (Tokyo, Japan). A TSK gel

PW precolumn (a hydrophilic metaacrylate polymer column) for sample clean-up (column I; 35 mm × 4.6 mm ID, particle size 10 μm; Tosoh, Tokyo, Japan) and a C18 STR ODS-II column as an analytical column (column II; 150 mm × 4.6 mm ID, particle size 5 μm; Shinwa Chemical Industry, Kyoto, Japan) were used.

2.5. Extraction procedure

IS (cisapride) 80 μL of 10 μg/ml, 1.0 ml NaOH (0.25 M) were added to 1.5 ml of plasma. The tubes were vortex-mixed for 10 s and 5 ml of toluene–chloroform (85:15, v/v) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 1700 × *g* for 10 min at 4 °C, and the organic phase was evaporated in vacuo at 45 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved with 750 μL of eluent A and used as an extract.

2.6. Chromatographic condition

Column-switching chromatographic condition was set based on our previous report [20]. A 0.5 ml portion of the extract was automatically injected into the HPLC system. Column-switching time schedule was set based on retention time of fluvoxamino acid in column I (about 12 min), retention time of achievement of maximal recovery of fluvoxamine from column I to column II (about 17 min) and retention time of appearance of interference peaks (about 20 min). From 0 to 11.5 min after the sample injection, fluvoxamine, fluvoxamino acid and IS were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (0.02 M, pH 4.6), acetonitrile and perchloric acid (60%) (92.4:7.5:0.1, v/v/v). Between 11.5 and 18.0 min after the injection, two analytes and IS retained on column I were eluted with a mobile phase (eluent B) of phosphate buffer (0.02 M, pH 4.6), acetonitrile and perchloric acid (60%) (62.4:37.5:0.1, v/v/v), and effluent from column I was switched to column II. Then fluvoxamine and fluvoxamino acid were separated on column II by eluting with eluent B (between 18.0 and 35.0 min). The flow rates of eluents A and B were 1.2 and 0.6 mL/min, respectively. The temperatures of column I and II were about 25 (room temperatures) and 30 °C, respectively. The peak was detected using a UV detector set at 254 nm. The peak area was used for the quantification of these two compounds.

3. Results and discussion

3.1. Chromatography

Effects of constitution ratio of perchloric acid (0, 0.1, 0.25 and 1.0%) in mobile phase on peak separation of fluvoxamine, fluvoxamino acid and IS were investigated, and 0.1% perchloric acid obtained optimal separation of these compounds.

Table 1
Extraction recovery of analytes from plasma (n = 6)

Analyte	Concentration added (ng/mL)	Recovery (%)	CV (%)
Fluvoxamine	1.2	94.8	0.77
	24.0	94.8	0.66
	96.0	94.9	0.71
Fluvoxamino acid	0.9	96.4	0.91
	18.0	96.7	0.90
	72.0	96.4	0.64

A mobile phase with the same pH was adjusted with phospholic acid as with 0.1% perchloric acid did not lead to separation of any these compounds, suggesting a specific effect of perchloric acid but not pH on this method. The chromatogram of an extracted blank plasma sample is shown in Fig. 2A. A representative chromatogram of an extracted blank plasma sample spiked with working aqueous solution containing fluvoxamine and fluvoxamino acid and cisapride (internal standard) is shown in Fig. 2B (2.4 and 1.6 ng/ml, respectively) and C (96 and 72 ng/ml, respectively). The chromatograms of extracted plasma samples obtained from a patient receiving fluvoxamine 100 mg/day did not show interference peaks (Fig. 2D). All compounds were well separated from each other and from the front of the solvent peaks. Plasma concentrations were 41.7 ng/ml for fluvoxamine and 44.8 ng/ml for fluvoxamino acid.

3.2. Recovery and linearity

Absolute recovery from plasma was calculated by comparing the peak areas of pure standards prepared in purified water, and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compound (n = 6 each). Recoveries and their CV values were determined at three different concentrations ranging from 1.2 to 96 ng/ml for fluvoxamine and from 0.9 to 72 ng/ml for fluvoxamino acid. Recoveries and their CV values were 94–96% with less than 1% for both compounds (Table 1). Calibration curves were linear over the concentrations range from 0.8 to 153.6 ng/ml (r > 0.998) for fluvoxamine and from 0.6 to 115.2 ng/ml for fluvoxamino acid (r > 0.999).

3.3. Sensitivity

Chloroform, toluene, *n*-hexane, heptane, isopropylether, diethylether and ethyl acetate and their combination were investigated. The combination of toluene–chloroform (85:15, v/v) was the highest sensitivity among these organic solvents because of flat baseline. The limit of detection was defined as analyte responses that were at least three times the response compared to blank response. The limits of detection of fluvoxamine and fluvoxamino acid were 0.4 and 0.3 ng/ml, respectively. The lowest standard on the calibration curve was defined as the limit of quantification as analyte peaks by which both compounds in blank plasma were identifiable,

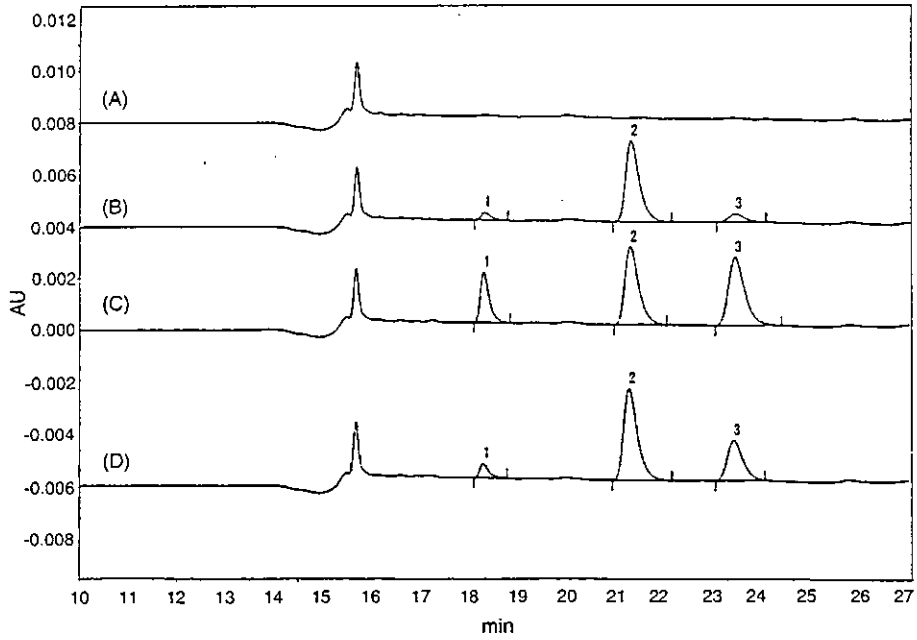

Fig. 2. Representative chromatogram of extracts of blank plasma (A) and extracts of plasma spiked with 2.4 ng/ml for fluvoxamine and 1.6 ng/ml for fluvoxamino acid (B) and 96 ng/ml for fluvoxamine and 72 ng/ml for fluvoxamino acid (C) and plasma from a patient (D). The peak 1 corresponds with fluvoxamino acid; peak 2 with cisapride; peak 3 with fluvoxamine.

discrete and reproducible with a precision of 20% and accuracy of 80–125%. The limits of quantification were 1.2 ng/ml for fluvoxamine and 0.9 ng/ml for fluvoxamino acid.

3.4. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with three different concentrations of two compounds. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and by analyzing a quality control for 6 days, respectively. These extracts underwent the same column-switching procedure. Intra-day coefficient variations (CVs) for fluvoxamine and fluvoxamino acid were less than 6.6 and 6.0%, respectively. Inter-day CVs for corresponding compounds were 6.3 and 6.5%, respectively (Table 2). Accuracy was expressed as mean percent error (relative error) [(measured plasma concentration − spiked concentration in plasma)/spiked concentration in plasma] × 100 (%) of each quality control plasma sample, while precision was quantitated by calculating intra- and inter CV values.

Table 2
Precision (CV) and accuracy (relative error) for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/mL)	Intra-day		Inter-day	
		CV (%)	Relative error (%)	CV (%)	Relative error (%)
Fluvoxamine	1.2	6.6	−5.8	6.3	−10.0
	24.0	2.1	−3.8	3.7	−5.1
	96.0	0.8	−1.3	1.0	−2.3
Fluvoxamino acid	0.9	6.0	−7.8	6.5	−10.9
	18.0	2.1	−5.6	2.8	−5.5
	72.0	1.1	−1.3	1.2	−2.4

3.5. Drug concentrations in human plasma

Fig. 3 shows the mean plasma concentration versus time curves of fluvoxamine and fluvoxamino acid after an oral administration of fluvoxamine (50 mg) in 10 subjects. The pharmacokinetic parameters of fluvoxamine and fluvoxamino acid are summarized in Table 3.

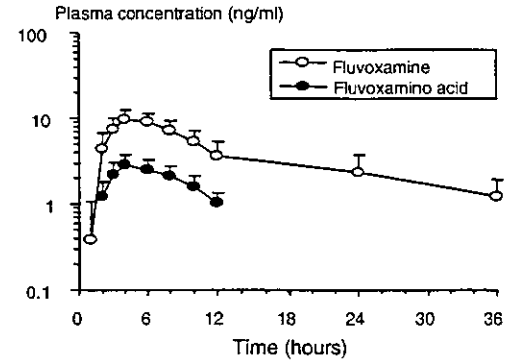

Fig. 3. Mean plasma concentration–time curves of fluvoxamine and fluvoxamino acid from 0.5 to 36 h in 10 healthy volunteers after a single-oral dose of fluvoxamine 50 mg. Error bars indicate standard deviation.

Table 3
Pharmacokinetic parameters of fluvoxamine and fluvoxamino acid after a oral 50 mg dose of fluvoxamine in 10 healthy volunteers

Parameters	Mean ± S.D.
Fluvoxamine	
C_{max} (ng/mL)	10.2 ± 2.7
T_{max} (h)	4.5 ± 1.0
$T_{1/2}$ (h)	14.1 ± 3.0
AUC(0–36) (ng h/mL)	131 ± 44
AUC(0–∞) (ng h/mL)	158 ± 64
Fluvoxamino acid	
C_{max} (ng/mL)	2.9 ± 0.8
T_{max} (h)	4.0 ± 0.8
AUC(0–12) (ng h/mL)	21.1 ± 5.9

AUC, area under plasma concentration–time curve; C_{max}, peak concentration; T_{max}, time to C_{max}; $T_{1/2}$, elimination half-life.

The sensitivity in the present simple HPLC method was superior to a previous method using HPLC analysis [16], enabling the monitoring of plasma concentrations of fluvoxamine up to 36 h and fluvoxamino acid up to 12 h after an administration of fluvoxamine 50 mg in all volunteers. Consequently, precise pharmacokinetic parameters were obtained from plasma concentration of fluvoxamine from 12 to 36 h after administration in elimination phase. These results can apply to clinical pharmacokinetic studies in patients receiving fluvoxamine treatment.

4. Conclusion

The new HPLC procedure described for simultaneous determination of fluvoxamine and fluvoxamino acid is suitable for routine analysis even though it is a little time consuming. Satisfactory validation data were achieved for linearity, precision and recovery. The limit of quantification obtained allows measurement of not only therapeutic concentration of fluvoxamine but also pharmacokinetic study using healthy volunteers.

References

[1] M.I. Wilde, G.L. Plosker, P. Benfield, Drugs 46 (1993) 895–924.
[2] H. Overmars, P.M. Scherpenisse, L.C. Post, Eur. J. Drug Metab. Pharmacokinet. 8 (1983) 269–280.
[3] G. Gerstenberg, T. Aoshima, T. Fukasawa, K. Yoshida, H. Takahashi, H. Higuchi, Y. Murata, R. Shimoyama, T. Ohkubo, T. Shimizu, K. Otani, Psychopharmacology 167 (2003) 443–448.
[4] G. Gerstenberg, T. Aoshima, T. Fukasawa, K. Yoshida, H. Takahashi, H. Higuchi, Y. Murata, R. Shimoyama, T. Ohkubo, T. Shimizu, K. Otani, Ther. Drug Monit. 25 (2003) 463–468.
[5] G.J. de Jong, J. Chromatogr. 183 (1880) 203–211.
[6] J. Pommery, M. Lhermitte, Biomed. Chromatogr. 3 (1989) 177–179.
[7] V. Van der Meersch-Mougeot, B. Diquet, J. Chromatogr. 567 (1991) 441–449.
[8] R.H. Pullen, A.A. Fatmi, J. Chromatogr. 574 (1992) 101–107.
[9] S. Hartter, H. Wetzel, C. Hiemke, Clin. Chem. 38 (1992) 2082–2086.
[10] S.H. Wong, H.R. Kranzler, S. DellaFera, R. Fernandes, Biomed. Chromatogr. 8 (1994) 278–282.
[11] L. Palego, D. Marazziti, L. Biondi, G. Giannaccini, N. Sarno, A. Armani, A. Lucacchini, G.B. Cassano, L. Dell'Osso, Ther. Drug Monit. 22 (2000) 190–194.
[12] A. Lucca, G. Gentilini, S. Lopez-Silva, A. Soldarini, Ther. Drug Monit. 22 (2000) 271–276.
[13] R. Skibinski, G. Misztal, Acta Pol. Pharm. 58 (2001) 97–100.
[14] G. Tournel, N. Houdret, V. Hedouin, M. Deveau, D. Gosset, M. Lhermitte, J. Chromatogr. B 761 (2001) 147–158.
[15] L. Labat, M. Deveaux, P. Dallet, J.P. Dubost, J. Chromatogr. B 773 (2002) 17–23.
[16] T. Ohkubo, R. Shimoyama, K. Otani, K. Yoshida, H. Higuchi, T. Shimizu, Anal. Sci. 19 (2003) 859–864.
[17] C. Duverneuil, G.L. de la Grandmaison, P. de Mazancourt, J.C. Alvarez, Ther. Drug Monit. 25 (2003) 565–573.
[18] K. Titier, N. Castaing, E. Scotto-Gomez, F. Pehourcq, N. Moore, M. Molimard, Ther. Drug Monit. 25 (2003) 581–587.
[19] J. van Harten, Clin. Pharmacokinet. 24 (1993) 203–220.
[20] N. Yasui-Furukori, Y. Inoue, T. Tateishi, J. Chromatogr. B 789 (2003) 239–245.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 374 (2005) 21–24
Neuroscience Letters
www.elsevier.com/locate/neulet

No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case–control population

Shinichi Saito[a,1], Masashi Ikeda[a,b,1], Nakao Iwata[b], Tatsuyo Suzuki[b], Tsuyoshi Kitajima[b], Yoshio Yamanouchi[b], Yoko Kinoshita[b], Nagahide Takahashi[a], Toshiya Inada[a,*], Norio Ozaki[a]

[a] *Department of Psychiatry and Psychobiology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[b] *Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan*

Received 3 September 2004; received in revised form 5 October 2004; accepted 6 October 2004

Abstract

ZDHHC8 is a new and attractive candidate for a schizophrenia-susceptibility factor. First, several lines of linkage studies showed that 22q11, on which ZDHHC8 is located, is a "hot" region. Second, fine linkage disequilibrium mapping revealed a significant association around ZDHHC8. Moreover, a very recent study reported that one single nucleotide polymorphism (SNP: rs175174) in ZDHHC8 might affect the splicing process, the ZDHHC8 knock-out mice showed the gender-specific phenotype, and the transmission disequilibrium test (TDT) using this SNP also showed significant association with human female schizophrenia. Thus, we attempted a replication study of this SNP using relatively large Japanese case–control samples (561 schizophrenics and 529 controls). No association was found between schizophrenia and controls even after dividing samples by gender. Because our sample size provided quite high power, ZDHHC8 may not play a major role in Japanese schizophrenia. And our results did not support the gender-specific effect of this SNP.

Keywords: Chromosome 22q11; Gender difference; Candidate gene

The 22q11 region (OMIM: #600850 SCZD4) is associated with increased risk for schizophrenia [2]. Two independent meta-analyses of linkage studies showed the linkage around 22q11 [1,5], although one negative result was also reported [8]. This chromosome region contains at least three genes, COMT [12], PRODH2 and DGCR6 [7], implicated as susceptibility genes for schizophrenia.

Recently, ZDHHC8 was reported as a new and attractive candidate gene on 22q11 from the evidence of a genetic association study and animal study [6,9]. In the initial genetic association study, Liu et al. showed that three single nucleotide polymorphisms (SNPs) in ZDHHC8 were associated with schizophrenia. One of these SNPs (rs175174), which was located in intron 4 of ZDHHC8, showed the most highly significant *P* value [6]. This intronic SNP seemed to modify ZDHHC8 expression by causing imperfect splicing, intron retention and reduced enzyme activity. In addition, *Zdhhc8* knockout mice had a gender-dependent dimorphic deficit in prepulse inhibition similar to schizophrenia and reactivity to the psychomimetic *N*-methyl-D-aspartate (NMDA) receptor blocker dizocilpine. In the light of these findings, the transmission disequilibrium test (TDT) divided samples according to gender differences, revealing that human female schizophrenia was significantly associated with this SNP [9]. Thus, we here provide a replication study of rs175174 in ZDHHC8 using Japanese case–control samples.

A total of 561 patients with schizophrenia (259 female; mean age ± standard deviation (S.D.) 49.6 ± 16.4 years: 302

* Corresponding author. Tel.: +81 52 744 2284; fax: +81 52 744 2293.
E-mail address: inada@med.nagoya-u.ac.jp (T. Inada).
[1] These authors contributed equally to this work.

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.10.015