trials or trials of pulse with prepulse at the three different intensities (PP trials) performed eight times for each condition. Block 3 was the same as block 1 to explore the habituation phenomenon in one of our previous publications (Takahashi et al., 2008). However, because habituation was not assessed in this study, Block 3 was not used for analysis of these data. All trials were presented in a fixed pseudorandom order, separated by inter-trial intervals of 15–25 s (20 s on average). The startle paradigm consisted of a total of 44 trials. The session lasted approximately 20 min, including 5 min acclimation to the background noise.

The following startle measures were examined: PPI82, PPI86, PPI90: prepulse inhibition at prepulse intensities of 82 dB, 86 dB, and 90 dB SPL, respectively. PPI for each prepulse intensity was computed as the percentage of magnitude reduction between PA and PP trials in block 2 by the formula: (1 – average eyeblink magnitude of startle response to PP trials in block 2/average eyeblink magnitude of startle response to PA trials in block 2) × 100.

Prior to data analyses, exclusion criteria were established for both trials and subject data. Trials were discarded if the voltage of their peak activity of the EMG within a latency window of 0–20 ms following startle eliciting stimulus onset was more than 30 microvolt. Subjects were excluded from further analyses as nonresponders if the voltages of their peak activity of the EMG within a latency window of 20–85 ms following startle eliciting stimulus onset were less than 30 microvolt in more than half of the trials in block 1. Analyses of PPI were not conducted if more than half of the PP trials at any prepulse intensity or PA trials in block 2 were discarded.

Upon arriving at the laboratory, each subject read and signed an informed consent form and completed a brief medical history questionnaire including demographic data. The subjects were informed about the general purpose of the study, about the stimuli and procedure, and that they could withdraw from the study at any time. Subjects were told that the experiment aimed to measure their reactivity to a number of noise bursts. There was no restriction on smoking intake, but we took care to avoid testing smokers within 30 min of smoking a cigarette, as this could potentially increase PPI (Kumari, Soni, & Sharma, 2001). Subjects were then seated in the testing room. During the task, the subjects were instructed to keep their eyes open and to maintain their gaze on a fixed point 100 cm away. Thereafter, the skin area at the electrode site was cleaned and the electrodes were attached. The door to the experimental chamber was closed.

Nineteen subjects were excluded from the analyses. One female subject could not stand the startle stimuli and did not complete the session. There were a total of 8 nonresponders. Ten subjects were excluded from analyses because their PPI were not evaluated according to the above exclusion criteria. There was one outlier, who was more than 3 standard deviations above or below the mean of all subjects, in cognitive/perceptual scores, and there was also another outlier in disorganization scores. We excluded these two outliers from further analyses. Thus, the final sample size was 79 (males $N = 33$, females $N = 46$; age [years]: $M = 38.5$, $SD = 10.7$, range 21–60). The percentage of smokers was higher in males than females (nonsmoker/smoker: males 22/11; females 39/7), although the difference did not reach statistical significance ($\chi^2(1) = 3.58$, $p = .101$, Fisher's exact test). Age did not differ significantly across sex (males: $M = 36.5$, $SD = 9.4$; females: $M = 39.8$, $SD = 11.4$; $t[77] = -1.36$, $p = .179$) and smoking status (nonsmokers: $M = 38.5$, $SD = 11.4$; smokers: $M = 38.3$, $SD = 7.7$; $t[41.2] = -.10$, $p = .922$). Those subjects excluded from the analyses did not differ significantly from the included subjects in demographic characteristics, such as age, sex distribution, smoking status, and also in the SPQ scores.

Statistical Analysis

None of the SPQ scores and startle measures was normally distributed based on the Shapiro–Wilkes W statistic ($p < .001$ for all SPQ scores; PPI82, $W = .963$, $p = .021$; PPI86, $W = .957$, $p = .009$; PPI90, $W = .969$, $p = .049$). Therefore, we performed nonparametric analyses. The Mann-Whitney U test was used for comparison of mean SPQ scores and startle measures. Within group differences in PPI across the three prepulse intensities were analyzed using the non-parametric Friedman χ^2 test. Spearman's rank order correlations examined the relationship of PPI to psychiatric symptoms. All p-values reported here were two-tailed. Statistical significance was considered when p-value was $< .05$. Statistical analyses were performed using SPSS Ver. 12 (SPSS Japan, Tokyo, Japan).

Results

Difference in Schizotypal Personality Questionnaire Scores and Startle Measures Across Sex and Smoking Status

The SPQ scores and startle measures of the subjects in the present study are shown in Table 1. Since sex and smoking status may affect startle measures (Abel, Waikar, Pedro, Hemsley, & Geyer, 1998; George, Termine, Sacco, Allen, Reutenauer, et al., 2006; Kumari, Aasen, & Sharma, 2004; Kumari, Checkley, & Gray, 1996; Kumari et al., 2001; Rissling, Dawson, Shell & Nuechterlein, 2007; Swerdlow, Auerbach, Monroe, Hartston, Geyer, & Braff, 1993; Swerdlow, Hartman, & Auerbach, 1997), and are also related to schizotypy (Badcock & Dragovic, 2006; Esterberg, Jones, Compton, & Walker, 2007; Kremen, Faraone, Toomey, Seidman, & Tsuang, 1998; Wan, Crawford, & Boutros, 2007), we assessed the difference in SPQ scores and startle measures across sex groups and smoking status. PPI differed significantly across the three prepulse intensities ($\chi^2(2) = 36.9$, $p < .01$), with PPI82 showing the lowest PPI, and PPI90 showing the highest PPI. This difference was also observed after separate analyses for sex (male: $\chi^2(2) = 25.9$, $p < .01$, female: $\chi^2(2) = 14.39$, $p < .01$), and smoking status (smoker: $\chi^2(2) = 9.33$, $p < .01$, nonsmoker: $\chi^2(2) = 30.33$, $p < .01$). As shown in Table 1, females had significantly decreased PPI for all three prepulse intensities compared to males, and SPQ scores did not differ significantly across sex. We did not find significant difference in SPQ scores or startle measures between smokers and nonsmokers (PPI82, $U = 442$, $p = .211$; PPI86, $U = 463$, $p = .315$; PPI90, $U = 514$, $p = .682$; the total SPQ score, $U = 469$, $p = .349$; cognitive/perceptual score, $U = 525$, $p = .776$; interpersonal score, $U = 463$, $p = .312$; disorganization score, $U = 484.5$, $p = .445$).

Relationship of Startle Measures to Schizotypy[1]

Figure 1 shows scatterplots of prepulse inhibition by scores on SPQ. PPI86 correlated negatively with the total SPQ score, $p = .002$, as well as with cognitive/perceptual scores, $p = .026$, and with interpersonal scores, $p = .003$. PPI90 also correlated negatively with the total SPQ score, $p = .020$, as well as with

[1]Since smoking status might have affected our results, we additionally investigated the relationship of startle measures and SPQ scores in nonsmokers. We found significant correlation between PPI86 and interpersonal scores in nonsmokers, rho = $-.268$, $p = .037$.

Table 1. *Scores on Schizotypal Personality Questionnaire and Startle Measures*

		All ($N = 79$)				Male ($N = 33$)		Female ($N = 46$)				
		M	*SD*	Skewness	Kurtosis	*M*	*SD*	*M*	*SD*	*U*	*p*	Effect size
Scores on SPQ	Total SPQ score	9.4	6.5	0.76	−0.21	9.2	7.1	9.5	6.1	703.5	0.580	0.057
	Cognitive/perceptual score	2.7	2.6	1.04	1.08	2.7	3.0	2.8	2.2	682.5	0.440	0.053
	Interpersonal score	4.6	3.8	1.00	0.46	4.5	3.7	4.7	3.9	725.0	0.734	0.075
	Disorganization score	2.6	2.3	0.60	−0.61	2.6	2.5	2.6	2.1	732.0	0.786	0.018
Startle measures	PPI82 (%)	27.2	27.2	−0.65	0.28	37.6	27.0	19.7	25.2	448.0	0.002	0.656
	PPI86 (%)	31.9	27.5	−0.38	−0.82	41.4	25.4	25.2	27.1	506.5	0.012	0.590
	PPI90 (%)	40.7	26.0	−0.39	−0.36	50.7	23.6	33.5	25.5	462.0	0.003	0.662

Note: SPQ: Schizotypal personality questionnaire; PPI82, PPI86, PPI90: prepulse inhibition of acoustic startle reflex in prepulse of 82 dB, 86 dB, and 90 dB, respectively. *U*: Mann-Whitney *U* test.

cognitive/perceptual scores, $p = .037$, and with interpersonal scores, $p = .048$. There was no other significant correlation between SPQ scores and PPI.

Since there was gender difference in PPI, we investigated the relationship of startle measures to SPQ scores separately for sex groups. In female subjects, PPI86 correlated negatively with the total SPQ score, rho = −.41, $p = .005$, with cognitive-perceptual scores, rho = −.30, $p = .042$, and with interpersonal scores, rho = −.41, $p = .005$, whereas PPI90 correlated negatively with the total SPQ score, rho = −.31, $p = .037$. However, we found no significant correlation between PPI and SPQ scores in male subjects.

Discussion

In this study, we investigated a possible association between PPI and SPQ scores in a sample of 79 healthy Japanese subjects. We found that the total SPQ score, cognitive/perceptual score, and interpersonal score correlated negatively with PPI86 and PPI90.

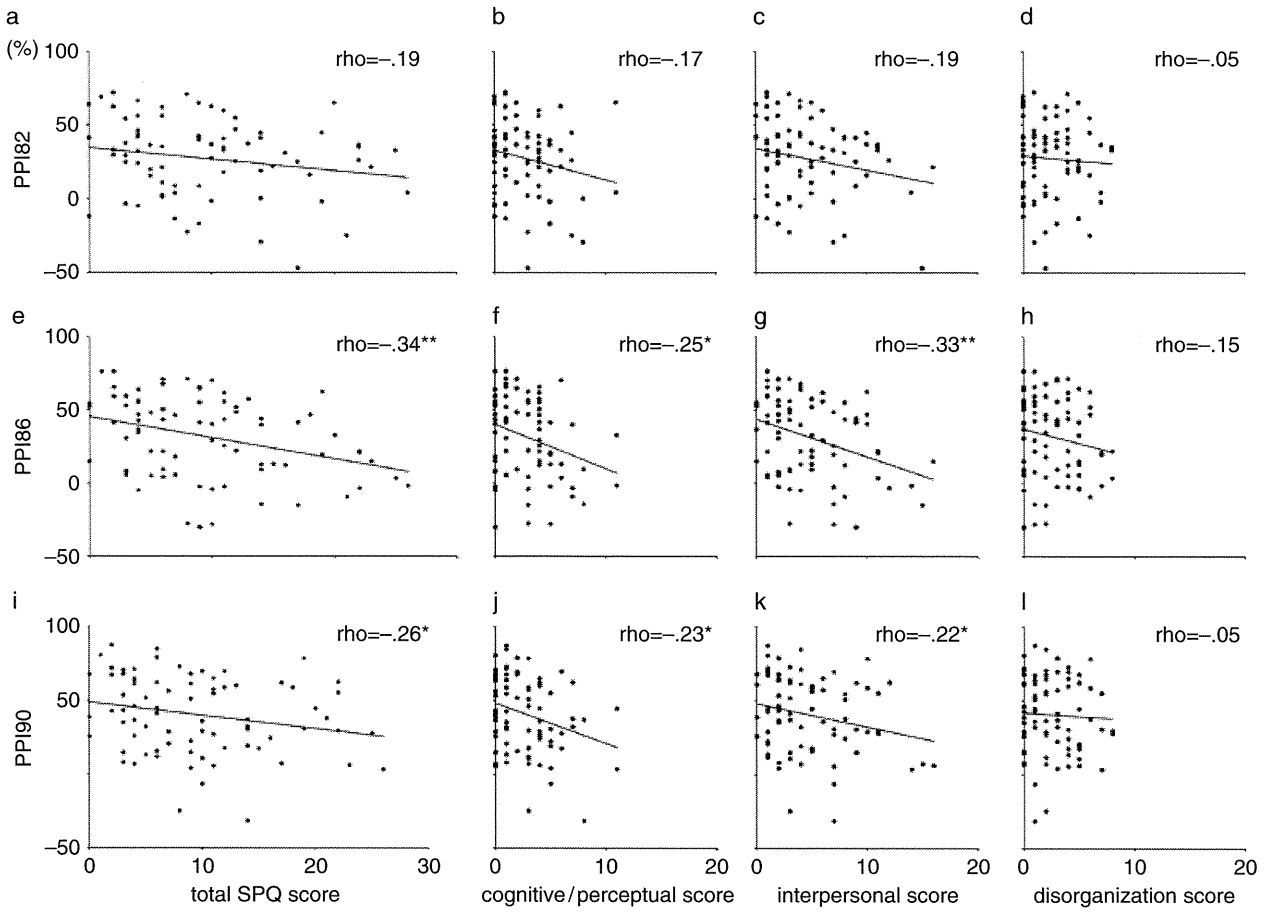

Figure 1. Scatterplot of prepulse inhibition by scores on schizotypal personality questionnaire ($N = 79$) Scatterplots of (a) PPI82 for the total SPQ score, (b) PPI82 for cognitive/perceptual score, (c) PPI82 for SPQ interpersonal score, (d) PPI82 for SPQ disorganization score, (e) PPI86 for the total SPQ score, (f) PPI86 for cognitive/perceptual score, (g) PPI86 for SPQ interpersonal score, (h) PPI86 for SPQ disorganization score, (i) PPI90 for the total SPQ score, (j) PPI90 for cognitive/perceptual score, (k) PPI90 for SPQ interpersonal score, and (l) PPI90 for SPQ disorganization score. Variables are rho. SPQ: Schizotypal personality questionnaire; PPI82, PPI86, PPI90: prepulse inhibition of acoustic startle reflex in prepulse of 82 dB, 86 dB, and 90 dB, respectively. Spearman's rank order correlations; *$p < .05$; **$p < .01$.

To our knowledge, this is the first study to investigate the relationship between PPI and schizotypy in non-Caucasian subjects. Since the profile of startle measures, which includes PPI, appears to be different in Caucasians compared with non-Caucasian populations (Hasenkamp et al., 2008; Swerdlow et al., 2005, 2007), the relationship between PPI and schizotypy might be different across race. However, we found that PPI86 and PPI90 negatively correlated with the total SPQ scores in healthy Japanese subjects. Our results indicate that the association of PPI to schizotypy might be detected across race. Further replication studies in non-Caucasian participants will be necessary to confirm this argument.

We used SPQ to assess schizotypy in relation to PPI. Although SPQ is a rather novel questionnaire to assess schizotypy, a recent study (Wuthrich & Bates, 2006) reported that SPQ scores showed good correlation with an established questionnaire of schizotypy, the Chapman schizotypy scales, which include the Chapman Magical Ideation (Eckblad & Chapman, 1983), Perceptual Aberration (Chapman, Chapman, & Raulin, 1978), and Revised Social Anhedonia (Eckblad, Chapman, Chapman, & Mishlove, 1982) scales. The SPQ assesses the nine diagnostic subscales of DSM-defined SPD, and the factor analytical study (Raine et al., 1994) showed that these nine diagnostic subscales for SPD can be reduced to three dimensions of schizotypy. Similarities between the three symptom factors of schizophrenia (Arndt, Alliger, & Andreasen, 1991; Bilder, Mukherjee, Rieder, & Pandurangi, 1985; Gruzelier, 1996; Liddle & Barnes, 1990) and the three SPQ dimensions of schizotypy suggested that analysis of the SPQ dimensions of schizotypy could be useful for evaluating the different components of schizotypy. In fact, the three-factor model (Raine et al., 1994) of SPQ has been suggested to underlie individual differences across samples of normal and schizophrenic patients (Rossi & Daneluzzo, 2002). Thus, the SPQ has been widely used to investigate the relationship of schizotypy to cognitive functions (Chen, Hsiao, Hsiao, & Hwu, 1998; Noguchi, Hori, & Kunugi, 2008) or to a psychophysiological index, such as P50 (Wan, Crawford, & Boutros, 2006, 2007; Wang, Miyazato, Hokama, Hiramatsu, & Kondo, 2004), P300 (Mannan, Hiramatsu, Hokama, & Ohta, 2001) or prefrontal activation patterns measured with near-infrared spectroscopy (Hori, Nagamine, Soshi, Okabe, Kim, & Kunugi, 2008; Hori, Ozeki, Terada, & Kunugi, 2008). By using SPQ, we could find a negative correlation between PPI and the trait of schizotypy.

Our result that females exhibited smaller PPI than males is consistent with findings of most previous PPI studies (Aasen, Kolli, & Kumari, 2005; Abel et al., 1998; Della Casa, Höfer, Weiner, & Feldon, 1998; Kumari et al., 2004; Swerdlow et al., 1993, 1995, 1997, 1999, 2006). In addition, the analysis of the data by sex difference indicated that association between SPQ scores and PPI remained significant among female subjects but not among male subjects. However, it is important to point out that, with the analytic approach used herein, we cannot ensure that reliable associations exist between sex and PPI in schizotypy in our data. Our results also showed a significant relationship between PPI and schizotypy exclusively for PPI86 and PPI90. This supports recent reports of a significant impact of stimulus SnR on PPI of ASR (Blumenthal, Noto, Fox, & Franklin, 2006; Franklin, Bowker, & Blumenthal, 2009; Franklin, Moretti, & Blumenthal, 2007). Of note, the correlation of PPI with schizotypy for PPI82 nearly reached statistical significance (the total SPQ score, $p = .103$; cognitive/perceptual scores, $p = .128$; interpersonal scores, $p = .097$). Thus, it is conceivable that an increase in sample size could also result in significant difference for this PPI intensity. Overall, although effects of sex and SnR on PPI may be interesting, the present study was not specifically designed to examine this issue but to assess the cross-cultural variability of the PPI–schizotypy relationship. Further studies will be necessary to clarify the effects of these factors on the relationship between PPI and schizotypy.

There are several limitations to the current study. First, we enrolled only healthy volunteers who have no family history of psychosis and were relatively mature-aged subjects. This might have restricted the range on the SPQ and influenced the relationship between PPI and SPQ scores. SPQ scores are reported high in relatives of patients with schizophrenia (Bora & Veznedaroglu, 2007) and are thought to become lower with increasing age (Badcock & Dragovic, 2006). Although SPQ scores in our sample were similar to those of mature, healthy populations (Chen et al., 1998), including those with participants without family history of psychiatric illness (Hori, Nagamine, et al., 2008; Hori, Ozeki, et al., 2008; Noguchi et al., 2008; Wang et al., 2004) (these studies have reported mean total SPQ scores ranging from 8.1 to 12.9), SPQ scores in our study were relatively smaller than those of previous studies on schizotypy, for instance, a study by Raine (1991) found a mean total SPQ score of 26.9. In addition, although symptom dimensions of schizotypy in relatives of patients with schizophrenia are reported in association with patient symptoms (Schürhoff, Laguerre, Szöke, Méary, & Leboyer, 2005), little is known about the relationships of symptom dimensions of schizotypy and schizophrenia. Further studies investigating the relationship between SPQ and PPI in relatives and non-relatives of patients with psychiatric disorders are needed.

Second, some SPQ scores, such as the interpersonal, cognitive-perceptual, and total SPQ scores, are associated with trait-anxiety (Braunstein-Bercovitz, 2000). Some previous studies (Duley, Hillman, Coombes, & Janelle, 2007; Franklin et al., 2009; Ludewig, Ludewig, Geyer, Hell, & Vollenweider, 2002) have reported a relationship between PPI and anxiety. Because we did not assess trait-anxiety of our subjects, the possibility that anxiety is more responsible for PPI than symptom dimension of schizotypy is not testable in this study. Future studies are needed to evaluate the association of PPI to psychiatric symptoms, including symptom dimension of schizotypy and anxiety.

Conclusion

In the present study, PPI correlated negatively with the trait of schizotypy in healthy Asian subjects.

REFERENCES

Aasen, I., Kolli, L., & Kumari, V. (2005). Sex effects in prepulse inhibition and facilitation of the acoustic startle response: Implications for pharmacological and treatment studies. *Journal of Psychopharmacology, 19*, 39–45.

Abel, K., Waikar, M., Pedro, B., Hemsley, D., & Geyer, M. (1998). Repeated testing of prepulse inhibition and habituation of the startle reflex: A study in healthy human controls. *Journal of Psychopharmacology, 12*, 330–337.

Anokhin, A. P., Heath, A. C., Myers, E., Ralano, A., & Wood, S. (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neuroscience Letters, 353*, 45–48.

American Psychiatric Association. (1987). *Diagnostic and Statistical Manual of Mental Disorders* (Rev. 3rd ed). Washington, DC: American Psychiatric Association.

Arndt, S., Alliger, R. J., & Andreasen, N. C. (1991). The distinction of positive and negative symptoms. The failure of a two-dimensional model. *The British Journal of Psychiatry, 158*, 317–322.

Badcock, J. C., & Dragovic, M. (2006). Schizotypal personality in mature adults. *Personality and Individual Differences, 40*, 77–85.

Bilder, R. M., Mukherjee, S., Rieder, R. O., & Pandurangi, A. K. (1985). Symptomatic and neuropsychological components of defect states. *Schizophrenia Bulletin, 11*, 409–419.

Blumenthal, T. D., Noto, J. V., Fox, M. A., & Franklin, J. C. (2006). Background noise decreases both prepulse elicitation and inhibition of acoustic startle blink responding. *Biological Psychology, 72*, 173–179.

Bora, E., & Veznedaroglu, B. (2007). Temperament and character dimensions of the relatives of schizophrenia patients and controls: The relationship between schizotypal features and personality. *European Psychiatry, 22*, 27–31.

Braff, D., Stone, C., Callaway, E., Geyer, M., Glick, I., & Bali, L. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology, 15*, 339–343.

Braff, D. L., Geyer, M. A., Light, G. A., Sprock, J., Perry, W., Cadenhead, K. S., et al. (2001). Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. *Schizophrenia Research, 49*, 171–178.

Braff, D. L., & Light, G. A. (2005). The use of neurophysiological endophenotypes to understand the genetic basis of schizophrenia. *Dialogues in Clinical Neuroscience, 7*, 125–135.

Braunstein-Bercovitz, H. (2000). Is the attentional dysfunction in schizotypy related to anxiety? *Schizophrenia Research, 46*, 255–267.

Cadenhead, K. S., & Braff, D. L. (2002). Endophenotyping schizotypy: A prelude to genetic studies within the schizophrenia spectrum. *Schizophrenia Research, 54*, 47–57.

Cadenhead, K. S., Geyer, M. A., & Braff, D. L. (1993). Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. *American Journal of Psychiatry, 150*, 1862–1867.

Cadenhead, K. S., Swerdlow, N. R., Shafer, K. M., Diaz, M., & Braff, D. L. (2000). Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: Evidence of inhibitory deficits. *The American Journal of Psychiatry, 157*, 1660–1668.

Chapman, L. J., Chapman, J. P., & Raulin, M. L. (1978). Body-image aberration in schizophrenia. *Journal of Abnormal Psychology, 87*, 399–407.

Chen, W. J., Hsiao, C. K., Hsiao, L. L., & Hwu, H. G. (1998). Performance of the Continuous Performance Test among community samples. *Schizophrenia Bulletin, 24*, 163–174.

Della Casa, V., Höfer, I., Weiner, I., & Feldon, J. (1998). The effects of smoking on acoustic prepulse inhibition in healthy men and women. *Psychopharmacology, 137*, 362–368.

Duley, A. R., Hillman, C. H., Coombes, S., & Janelle, C. M. (2007). Sensorimotor gating and anxiety: Prepulse inhibition following acute exercise. *International Journal of Psychophysiology, 64*, 157–164.

Eckblad, M., & Chapman, L. J. (1983). Magical ideation as an indicator of schizotypy. *Journal of Consulting and Clinical Psychology, 51*, 215–225.

Eckblad, M. L., Chapman, L. J., Chapman, J. P., & Mishlove, M. (1982). Revised social anhedonia scale. Unpublished manuscript.

Esterberg, M. L., Jones, E. M., Compton, M. T., & Walker, E. F. (2007). Nicotine consumption and schizotypy in first-degree relatives of individuals with schizophrenia and non-psychiatric controls. *Schizophrenia Research, 97*, 6–13.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1997). *Structured clinical interview for DSM-IV axis I disorders, research version, non-patients edition (SCID-I/NP)*. New York: Biometrics Research Dept., New York State Psychiatric Institute.

Franklin, J. C., Bowker, K. B., & Blumenthal, T. D. (2009). Anxiety and prepulse inhibition of acoustic startle in a normative sample: The importance of signal-to-noise ratio. *Personality and Individual Differences, 46*, 369–373.

Franklin, J. C., Moretti, N. A., & Blumenthal, T. D. (2007). Impact of stimulus signal-to-noise ratio on prepulse inhibition of acoustic startle. *Psychophysiology, 44*, 339–342.

George, T. P., Termine, A., Sacco, K. A., Allen, T. M., Reutenauer, E., Vessicchio, J. C., et al. (2006). A preliminary study of the effects of cigarette smoking on prepulse inhibition in schizophrenia: Involvement of nicotinic receptor mechanisms. *Schizophrenia Research, 87*, 307–315.

Gruzelier, J. H. (1996). The factorial structure of schizotypy: Part I. Affinities with syndromes of schizophrenia. *Schizophrenia Bulletin, 22*, 611–620.

Hasenkamp, W., Norrholm, S. D., Green, A., Lewison, B., Boshoven, W., Keyes, M., et al. (2008). Differences in startle reflex and prepulse inhibition in European-Americans and African-Americans. *Psychophysiology, 45*, 876–882.

Hori, H., Nagamine, M., Soshi, T., Okabe, S., Kim, Y., & Kunugi, H. (2008). Schizotypal traits in healthy women predict prefrontal activation patterns during a verbal fluency task: A near-infrared spectroscopy study. *Neuropsychobiology, 57*, 61–69.

Hori, H., Ozeki, Y., Terada, S., & Kunugi, H. (2008). Functional near-infrared spectroscopy reveals altered hemispheric laterality in relation to schizotypy during verbal fluency task. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32*, 1944–1951.

Kremen, W. S., Faraone, S. V., Toomey, R., Seidman, L. J., & Tsuang, M. T. (1998). Sex differences in self-reported schizotypal traits in relatives of schizophrenic probands. *Schizophrenia Research, 34*, 27–37.

Kumari, V., Aasen, I., & Sharma, T. (2004). Sex differences in prepulse inhibition deficits in chronic schizophrenia. *Schizophrenia Research, 69*, 219–235.

Kumari, V., Checkley, S. A., & Gray, J. A. (1996). Effect of cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. *Psychopharmacology, 128*, 54–60.

Kumari, V., Das, M., Zachariah, E., Ettinger, U., & Sharma, T. (2005). Reduced prepulse inhibition in unaffected siblings of schizophrenia patients. *Psychophysiology, 42*, 588–594.

Kumari, V., Soni, W., & Sharma, T. (2001). Influence of cigarette smoking on prepulse inhibition of the acoustic startle response in schizophrenia. *Human Psychopharmacology, 16*, 321–326.

Kumari, V., Toone, B., & Gray, J. A. (1997). Habituation and prepulse inhibition of the acoustic startle reflex: Effects of smoking status and psychosis-proneness. *Personality and Individual Differences, 23*, 183–191.

Kunugi, H., Tanaka, M., Hori, H., Hashimoto, R., Saitoh, O., & Hironaka, N. (2007). Prepulse inhibition of acoustic startle in Japanese patients with chronic schizophrenia. *Neuroscience Research, 59*, 23–28.

Liddle, P. F., & Barnes, T. R. (1990). Syndromes of chronic schizophrenia. *The British Journal of Psychiatry, 157*, 558–561.

Ludewig, S., Ludewig, K., Geyer, M. A., Hell, D., & Vollenweider, F. X. (2002). Prepulse inhibition deficits in patients with panic disorder. *Depression and Anxiety, 15*, 55–60.

Mannan, M. R., Hiramatsu, K., Hokama, H., & Ohta, H. (2001). Abnormalities of auditory event-related potentials in students with schizotypal personality disorder. *Psychiatry and Clinical Neurosciences, 55*, 451–457.

Noguchi, H., Hori, H., & Kunugi, H. (2008). Schizotypal traits and cognitive function in healthy adults. *Psychiatry Research, 161*, 162–169.

Raine, A. (1991). The SPQ: A scale for the assessment of schizotypal personality based on DSM-III-R criteria. *Schizophrenia Bulletin, 17*, 555–564.

Raine, A., Reynolds, C., Lencz, T., Scerbo, A., Triphon, N., & Kim, D. (1994). Cognitive-perceptual, interpersonal, and disorganized features of schizotypal personality. *Schizophrenia Bulletin, 20*, 191–201.

Rissling, A. J., Dawson, M. E., Schell, A. M., & Nuechterlein, K. H. (2007). Effects of cigarette smoking on prepulse inhibition, its attentional modulation, and vigilance performance. *Psychophysiology, 44*, 627–634.

Rossi, A., & Daneluzzo, E. (2002). Schizotypal dimensions in normals and schizophrenic patients: A comparison with other clinical samples. *Schizophrenia Research, 54*, 67–75.

Schürhoff, F., Laguerre, A., Szöke, A., Méary, A., & Leboyer, M. (2005). Schizotypal dimensions: Continuity between schizophrenia and bipolar disorders. *Schizophrenia Research, 80*, 235–242.

Simons, R. F., & Giardina, B. D. (1992). Reflex modification in psychosis-prone young adults. *Psychophysiology, 29*, 8–16.

Someya, T., Sasaki, T., & Takahashi, S. (1994) Reliability and validity of schizotypal personality questionnaire (in Japanese). *The Proceeding of the 32nd Scientific Meeting of the University Health Care in Japan,* 286–290.

Swerdlow, N. R., Auerbach, P., Monroe, S. M., Hartston, H., Geyer, M. A., & Braff, D. L. (1993). Men are more inhibited than women by weak prepulses. *Biological Psychiatry, 34*, 253–260.

Swerdlow, N. R., Filion, D., Geyer, M. A., & Braff, D. L. (1995). "Normal": personality correlates of sensorimotor, cognitive, and visuospatial gating. *Biological Psychiatry, 37*, 286–299.

Swerdlow, N. R., Hartman, P. L., & Auerbach, P. P. (1997). Changes in sensorimotor inhibition across the menstrual cycle: Implications for neuropsychiatric disorders. *Biological Psychiatry, 41*, 452–460.

Swerdlow, N. R., Geyer, M. A., Hartman, P. L., Sprock, J., Auerbach, P. P., Cadenhead, K., et al. (1999). Sex differences in sensorimotor gating of the human startle reflex: All smoke? *Psychopharmacology, 146*, 228–232.

Swerdlow, N. R., Light, G. A., Cadenhead, K. S., Sprock, J., Hsieh, M. H., & Braff, D. L. (2006). Startle gating deficits in a large cohort of patients with schizophrenia: Relationship to medications, symptoms, neurocognition, and level of function. *Archives of General Psychiatry, 63*, 1325–1335.

Swerdlow, N. R., Sprock, J., Light, G. A., Cadenhead, K., Calkins, M. E., Dobie, D. J., et al. (2007). Multi-site studies of acoustic startle and prepulse inhibition in humans: Initial experience and methodological considerations based on studies by the Consortium on the Genetics of Schizophrenia. *Schizophrenia Research, 92*, 237–251.

Swerdlow, N. R., Talledo, J. A., & Braff, D. L. (2005). Startle modulation in Caucasian-Americans and Asian-Americans: A prelude to genetic/endophenotypic studies across the 'Pacific Rim'. *Psychiatric Genetics, 5*, 61–65.

Takahashi, H., Iwase, M., Ishii, R., Ohi, K., Fukumoto, M., Azechi, M., et al. (2008). Impaired prepulse inhibition and habituation of acoustic startle response in Japanese patients with schizophrenia. *Neuroscience Research, 62*, 187–194.

Turetsky, B. I., Calkins, M. E., Light, G. A., Olincy, A., Radant, A. D., & Swerdlow, N. R. (2007). Neurophysiological endophenotypes of schizophrenia: The viability of selected candidate measures. *Schizophrenia Bulletin, 33*, 69–94.

Wan, L., Crawford, H. J., & Boutros, N. (2007). Early and late auditory sensory gating: Moderating influences from schizotypal personality, tobacco smoking status, and acute smoking. *Psychiatry Research, 151*, 11–20.

Wan, L., Crawford, H. J., & Boutros, N. (2006). P50 sensory gating: Impact of high vs. low schizotypal personality and smoking status. *International Journal of Psychophysiology, 60*, 1–9.

Wang, J., Miyazato, H., Hokama, H., Hiramatsu, K., & Kondo, T. (2004). Correlation between P50 suppression and psychometric schizotypy among non-clinical Japanese subjects. *International Journal of Psychophysiology, 52*, 147–157.

Wuthrich, V. M., & Bates, T. C. (2006). Confirmatory factor analysis of the three-factor structure of the schizotypal personality questionnaire and Chapman schizotypy scales. *Journal of Personality Assessment, 87*, 292–304.

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SHORT COMMUNICATION

A two-stage case–control association study of the dihydropyrimidinase-like 2 gene (*DPYSL2*) with schizophrenia in Japanese subjects

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We examined the association of schizophrenia (SCZ) and dihydropyrimidinase-like 2 (*DPYSL2*), also known as collapsin response mediator protein 2, which regulates axonal growth and branching. We genotyped 20 tag single nucleotide polymorphisms (SNPs) in 1464 patients and 1310 controls. There were two potential associations in a screening population of 384 patients and 384 controls (rs2585458: *P*=0.046, rs4733048: *P*=0.014). However, we could not replicate these associations in a confirmatory population of 1080 patients and 926 controls (rs2585458: *P*=0.39, rs4733048: *P*=0.70) or a joint analysis (rs2585458: *P*=0.72, rs4733048: *P*=0.10). We conclude that *DPYSL2* does not have a major function in SCZ in Japanese subjects.
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Keywords: case–control study; *DPYSL2*; imputation; Japanese subjects; neuronal polarity; schizophrenia

INTRODUCTION

Schizophrenia (SCZ) is a severe debilitating neuropsychiatric disorder that affects ~1% of the general population. Family, twin and adoption studies support a substantial genetic contribution to SCZ, but its etiology remains unclear.[1] Moreover, irregularities consistent with abnormal brain development, including faulty neuronal migration and altered spatial neuronal arrangement have been reported in SCZ. Together with behavioral, neuromotor and other functional abnormalities that occur in childhood and predict SCZ, such as low IQ, poor motor skills and poor development of language and social skills, these morphological findings indicate a developmental origin for SCZ.[2,3]

Dihydropyrimidinase-like 2 (DPYSL2), also known as collapsin response mediator protein 2, is involved in the regulation of axon formation during neuronal polarization.[4,5] Overexpression of *Dpysl2* induces the formation of multiple axons and can alter an established dendrite to become an axon, indicating that overexpressed *Dpysl2* confers axonal identity not only on immature neurites but also on established dendrites. These observations indicate that DPYSL2 has a crucial function in axon formation of hippocampal neurons, thereby establishing and maintaining neuronal polarity. DPYSL2 interacts with tubulin heterodimers and promotes microtubule assembly *in vitro*. Thus, DPYSL2 seems to promote neurite elongation and axon specification by regulating microtubule assembly, endocytosis of adhesion molecules and reorganization of actin filaments.[6]

DPYSL2 is located on chromosome 8p21.2. This region has been reported as positive in meta-analysis of genome-wide linkage studies of SCZ.[7] One study showed that chromosomal 8p is a potential hub for developmental neuropsychiatric disorders and contains 21 genes (ADRA1A, ARHGEF10, CHRNA2, CHRNA6, CHRNB3, DKK4, DPYSL2, EGR3, FGF17, FGF20, FGFR1, FZD3, LDL, NAT2, NEF3, NRG1, PCM1, PLAT, PPP3CC, SFRP1 and VMAT1/SLC18A1) that are likely to contribute to the developmental neuropsychiatric disorders (that is SCZ, autism, bipolar disorder and depression) and neurodegenerative disorders (Parkinson's and Alzheimer's disease).[8] *DPYSL2* is involved in neuropsychiatric disorders's biology and clearly associated with SCZ and probably with bipolar disorder. The expression of *DPYSL2* in the hippocampus is increased in SCZ patients. Expression of the dihydropyrimidinase-related protein 2 in Down's syndrome and Alzheimer's disease brain is downregulated at the mRNA and dysregulated at the protein level. Several clinical studies have described a variety of neurodevelopmental abnormalities in subjects with defects of *DPYSL2*. *DPYSL2* is a marker for escitalopram resistance in stress model of depression. *DPYSL2* was reported to be a SCZ susceptibility gene in Japanese subjects.[9] However, the results of replication studies using smaller sets of markers have been inconsistent.[10,11] Therefore, to assess whether *DPYSL2* has a function in vulnerability to SCZ, we conducted a two-stage case–control association study in a Japanese population.

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Table 1 Allele frequencies of the 20 SNPs of *DPYSL2* in the screening population

			First set						
			Single marker (allele-wise)						
dbSNP	*Physical position*	*Minor allele*	*Cases*[a]	*Controls*[a]	*P*[b]	*OR*	*L95*[c]	*U95*[c]	*pHWE*[d]
rs327233	26500769	G	0.27	0.31	0.09	0.83	0.66	1.03	0.72
rs327232	26511632	T	0.20	0.24	0.09	0.81	0.64	1.03	0.48
rs13249543	26519261	G	0.40	0.39	0.81	1.03	0.84	1.26	0.59
rs2585458	26523749	A	0.09	0.13	**0.046**	0.72	0.52	1.00	0.24
rs182748	26527132	C	0.50	0.49	0.68	1.04	0.85	1.27	0.04
rs17321828	26527954	A	0.15	0.12	0.11	1.28	0.95	1.72	1.00
rs4733013	26528572	A	0.34	0.37	0.20	0.87	0.71	1.08	0.08
rs371255	26530311	A	0.25	0.25	0.86	0.98	0.78	1.23	0.04
rs403185	26532638	A	0.39	0.36	0.28	1.12	0.91	1.38	0.05
rs7829347	26536534	C	0.09	0.07	0.17	1.29	0.89	1.86	1.00
rs327221	26536952	A	0.10	0.12	0.12	0.77	0.56	1.07	0.48
rs4733033	26545142	C	0.38	0.41	0.20	0.88	0.71	1.07	0.20
rs327217	26546233	C	0.10	0.12	0.20	0.81	0.58	1.12	0.45
rs1972921	26551657	T	0.21	0.25	0.10	0.82	0.64	1.04	0.49
rs1442337	26552304	A	0.15	0.16	0.87	0.98	0.74	1.29	1.00
rs4733048	26555006	C	0.17	0.22	**0.014**	0.73	0.56	0.94	0.36
rs708621	26566709	C	0.33	0.31	0.39	1.10	0.89	1.37	0.81
rs11863	26570233	T	0.28	0.26	0.41	1.10	0.88	1.38	0.79
rs920633	26570696	T	0.46	0.44	0.50	1.07	0.88	1.31	0.25
rs17666	26571375	C	0.15	0.14	0.92	1.02	0.76	1.35	0.67

Abbreviations: OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Minor allele frequency.
[b]Fisher exact test.
[c]Lower (L) and upper (U) 95% confidence intervals.
[d]Hardy–Weinberg equilibrium *P*-value in controls.
Bold values indicate that they are lower than *P*-value 0.05.

MATERIALS AND METHODS

Participants

This study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from each participant. Patients were included in the study if they (1) met DSM-IV criteria for SCZ, (2) were physically healthy and (3) had no mood disorders, substance abuse, neurodevelopmental disorders, epilepsy or known mental retardation. Consensus diagnoses were made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of unstructured interviews with patients and families and review of medical records. The rate of the samples excluded due to the loss of the consensus was <5%. Controls were selected from the general population who had no history of mental disorders based on self-administered questionnaire during sample inclusion step, and based on unstructured diagnostic interview done by a experienced psychiatrist during the blood collection step. All subjects were unrelated to each other, living in the central area of the main land of Japan and self-identified as Japanese population.

Participants consisted of 1464 unrelated Japanese patients with SCZ (age 44.8 ± 15.0 years (mean ± s.d.), male 52.6%) and 1310 unrelated healthy controls (age 36.0 ± 13.7 years (mean ± s.d.), male 51.0%). In a screening population, participants consisted of 384 unrelated Japanese patients with SCZ (age 50.5 ± 15.1 years (mean ± s.d.), male 52.6%) and 384 unrelated healthy controls (age 38.1 ± 14.2 years (mean ± s.d.), male 60.3%). In a confirmatory population, participants consisted of 1080 unrelated Japanese patients with SCZ (age 42.7 ± 14.4 years (mean ± s.d.), male 49.8%) and 926 unrelated healthy controls (age 35.1 ± 13.36 years (mean ± s.d.), male 55.4%). Characterization of general samples and sampling procedures are available elsewhere.[12]

Genotyping and data analysis

In the screening population of 384 patients and 384 controls, we examined 20 single nucleotide polymorphisms (SNPs) including the positive SNP (rs17666) in an earlier study of a Japanese population.[9] The 20 SNPs were selected by

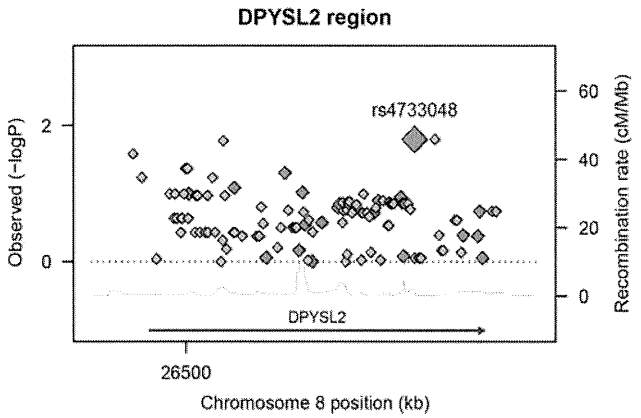

Figure 1 Allele *P*-values of the typed and the imputed SNPs. Red represents typed SNPs and gray represents imputed SNPs. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Haploview ver4.1 program[13] according to the HapMap database (release no. 24, population: Japanese in Tokyo, minor allele frequency >10%). SNP tagging criteria were based on minor allele frequency (>10%) and correlation coefficient (r^2) between loci (>0.8) as reported in the HapMap database. We genotyped all SNPs by TaqMan assay (Applied Biosystems Japan, Tokyo, Japan). For quality control, we checked the deviation from the Hardy–Weinberg equilibrium, the sample-wise call rate >95% and the SNP-wise call rate >95%. A power calculation was done by Power Calculator for Genetics Studies.[14] We did statistical calculations using PLINK ver2.05.[15] The Fisher exact test was used to compare allele frequencies between patients and controls. Significance level was set at $P<0.05$. As tagging SNPs were selected based on r^2,

Table 2 Allele frequencies of the two SNPs of *DPYSL2*

			Second set							Joint analysis				
			Single marker (allele-wise)							Single marker (allele-wise)				
dbSNP	*Physical position*	*Minor allele*	*Cases*[a]	*Controls*[a]	*P*[b]	*OR*	*L95*[c]	*U95*[c]	*pHWE*[d]	*P*[e]	*OR*	*L95*[c]	*U95*[c]	*P_BD*[f]
rs2585458	26523749	A	0.12	0.11	0.39	1.09	0.89	1.34	0.86	0.72	0.97	0.82	1.15	0.03
rs4733048	26555006	C	0.18	0.19	0.70	0.97	0.82	1.14	0.91	0.10	0.89	0.77	1.02	0.06

Abbreviations: OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Minor allele frequency.
[b]Fisher exact test.
[c]95% confidence intervals.
[d]Hardy–Weinberg equilibrium *P*-value in controls.
[e]Cochran–Mantel–Haenszel test.
[f]Breslow-day test.

we included imputation as an exploratory method to compute genotypes of SNPs that were not genotyped.[16] The starting point of imputation methods is a reference data set such as the HapMap, in which a large set of SNPs is being genotyped. The underlying assumption is that the reference samples, the cases and the controls are all sampled from the same population. Under this assumption, the three populations share the same linkage disequilibrium structure and the same haplotype distribution for every set of SNPs. Thus, the structure of the linkage disequilibrium in the reference population, in conjunction with the structure of the linkage disequilibrium of the observed SNPs within both the cases and the controls, can be used to impute the alleles of a hidden SNP. Imputed SNPs can then be tested for association using an appropriate statistical test. The advantage of imputing untyped SNPs is that the coverage of common variants within the locus of interest can be enhanced, boosting the statistical power and reducing type 2 errors in the screening population. The MACH program was used to calculate the genotypic prediction of 96 untyped SNPs using directly typed information from the 20 SNPs in the screening scan and the HapMap database (recombination map and haplotype data for the Japanese/Chinese population, release 24; phase II).[17] The MACH program was recently reported to have similar imputation accuracy rates to IMPUTE and to outperform fastPHASE, PLINK and Beagle.[18] The targeted region of imputation was limited to the *DPYSL2* locus.

RESULTS

We identified two association signals by PLINK ver2.05 between two SNPs and SCZ before multiple comparisons in the screening population (rs2585458: P=0.046, rs4733048: P=0.014) (Table 1). These two SNPs are located in intron. We imputed the genotype of 96 untyped SNPs using the MACH program and calculated the allelic *P*-value of each imputed SNP by Haploview ver4.1 (Figure 1). The lowest *P*-value in imputation was 0.016 at both typed rs4733048 and imputed rs4076071. To confirm the potential associations of these two SNPs and SCZ, we genotyped the two SNPs in a confirmatory population. However, we could not replicate these associations in the confirmatory population of 1080 patients and 926 controls (rs2585458: P=0.39, rs4733048: P=0.70) or in a joint analysis (rs2585458: P=0.72, rs4733048: P=0.10) (Table 2).

DISCUSSION

In this study, we investigated the association between 20 SNPs of *DPYSL2* and SCZ in a Japanese population. We did not observe significant associations between *DPYSL2* and SCZ. The positive association observed in the screening population might be due to a type 1 error. After Bonferroni correction, all SNPs in the screening population were negative. Sample size of this study is larger than earlier studies and we used an imputation method, boosting statistic power, so a type 2 error seems unlikely. However, other SNP might be associated with SCZ in the screening sample. Furthermore, we have conducted meta-analysis of data reported previously for rs17666,[9–11] but we could not detect association (fixed model OR_{ci95}=0.877–1.175, P=0.843). However, this study may not have sufficient power to detect associations between SNPs with smaller effects and SCZ. It should be noted that a larger sample is required for the detection of a smaller effect. The present sample has statistical power >0.8 for the detection of the role of the polymorphism with a minor allele frequency of 0.1, when the genotype relative risk is 1.35. As all participants in this study were of Japanese descent and recruited from the main island of Japan, the likelihood of population stratification is low.[19]

There are several limitations in our study. The first limitation in our association study is that cases and controls were not matched in terms of age. In other words, the controls may develop SCZ at some point in life, as they were significantly younger than cases. This might affect the statistic power. The second limitation in our study is that our study design was based on the common disease common variant hypothesis, so we applied minor allele frequency >10% when we selected the 20 tagSNPs. It is difficult to evaluate the association between rare variants and SCZ in our study. In conclusion, this study did not show evidence for the association of *DPYSL2* with SCZ in the Japanese population. *DPYSL2* may not have a major function in genetic susceptibility to SCZ.

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1 Harrison, P. J. & Weinberger, D. R. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol. Psychiatry* **10**, 40–68 image 45 (2005).
2 Benes, F. M., Davidson, J. & Bird, E. D. Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. *Arch. Gen. Psychiatry* **43**, 31–35 (1986).
3 Crow, T. J. A. Darwinian approach to the origins of psychosis. *Br. J. Psychiatry* **167**, 12–25 (1995).
4 Inagaki, N., Chihara, K., Arimura, N., Menager, C., Kawano, Y., Matsuo, N. *et al.* CRMP-2 induces axons in cultured hippocampal neurons. *Nat. Neurosci.* **4**, 781–782 (2001).
5 Conde, C. & Caceres, A. Microtubule assembly, organization and dynamics in axons and dendrites. *Nat. Rev. Neurosci.* **10**, 319–332 (2009).
6 Arimura, N. & Kaibuchi, K. Neuronal polarity: from extracellular signals to intracellular mechanisms. *Nat. Rev. Neurosci.* **8**, 194–205 (2007).

7 Ng, M. Y., Levinson, D. F., Faraone, S. V., Suarez, B. K., DeLisi, L. E., Arinami, T. *et al.* Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol. Psychiatry* **14,** 774–785 (2009).
8 Tabares-Seisdedos, R. & Rubenstein, J. L. Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. *Mol. Psychiatry* **14,** 563–589 (2009).
9 Nakata, K., Ujike, H., Sakai, A., Takaki, M., Imamura, T., Tanaka, Y. *et al.* The human dihydropyrimidinase-related protein 2 gene on chromosome 8p21 is associated with paranoid-type schizophrenia. *Biol. Psychiatry* **53,** 571–576 (2003).
10 Hong, L. E., Wonodi, I., Avila, M. T., Buchanan, R. W., McMahon, R. P., Mitchell, B. D. *et al.* Dihydropyrimidinase-related protein 2 (DRP-2) gene and association to deficit and nondeficit schizophrenia. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **136B,** 8–11 (2005).
11 Zhao, X., Tang, R., Xiao, Z., Shi, Y., Feng, G., Gu, N. *et al.* An investigation of the dihydropyrimidinase-like 2 (DPYSL2) gene in schizophrenia: genetic association study and expression analysis. *Int. J. Neuropsychopharmacol.* **9,** 705–712 (2006).
12 Ikeda, M., Takahashi, N., Saito, S., Aleksic, B., Watanabe, Y., Nunokawa, A. *et al.* Failure to replicate the association between NRG1 and schizophrenia using Japanese large sample. *Schizophr. Res.* **101,** 1–8 (2008).
13 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics (Oxford, England)* **21,** 263–265 (2005).
14 Skol, A. D., Scott, L. J., Abecasis, G. R. & Boehnke, M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat. Genet.* **38,** 209–213 (2006).
15 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559–575 (2007).
16 Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39,** 906–913 (2007).
17 Li, Y., Willer, C., Sanna, S. & Abecasis, G. Genotype imputation. *Annu. Rev. Genomics. Hum. Genet.* **10,** 387–406 (2009).
18 Pei, Y. F., Li, J., Zhang, L., Papasian, C. J. & Deng, H. W. Analyses and comparison of accuracy of different genotype imputation methods. *PloS One* **3,** e3551 (2008).
19 Yamaguchi-Kabata, Y., Nakazono, K., Takahashi, A., Saito, S., Hosono, N., Kubo, M. *et al.* Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am. J. Hum. Genet.* **83,** 445–456 (2008).

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DEBATE Open Access

Laughter and humor as complementary and alternative medicines for dementia patients

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Abstract

Background: The number of dementia patients has increased worldwide, with an estimated 13.7 million dementia patients in the Asia Pacific region alone. This number is expected to increase to 64.6 million by the year 2050.

Discussion: As a result of advances in research, there several pharmacological therapies available for the treatment of dementia patients. However, current treatments do not suppress the disease process and cannot prevent dementia, and it will be some time before these goals are realized. In the meantime, complementary and alternative medicine (CAM) is an important aspect in the treatment of dementia patients to improve their quality of life throughout the long course of the disease. Considering the individuality of dementia patients, applicability of laughter and humor therapy is discussed. Even though there are many things that need to be elucidated regarding the mechanisms underlying the beneficial effects of laughter and humor, both may be good CAM for dementia patients if they are applied carefully and properly.

Summary: In this debate article, the physiological basis and actual application of laughter and humor in the treatment of dementia patients are presented for discussion on the applicability to dementia patients.

Background

Because of the rapidly increasing elderly population, the need for psychogeriatric services will increase in coming years. In particular, a faster aging of the population has been observed in Asian countries compared with that in Western countries. The World Health Organization has proposed that for a society to be called 'aging', the proportion of elderly citizens (aged 65 years and older) must be 7%. Once this proportion reaches 14%, a society becomes an 'aged society' [1]. It took 24 years for Japan to move from an aging society (in 1970) to an aged society (in 1994); in comparison, in most Western countries this process takes 60-120 years [1]. Korea is expected to become an aged society by 2019, only 19 years after becoming an aging society (2000).

Considerable progress has been made in psychogeriatric services as a result of increased knowledge of brain science, neuroscience, molecular genetics, brain imaging, and many other new technologies [2]. The mechanisms underlying the cognitive impairment in dementia patients are now understood because of findings from brain science and neuropsychological investigations [3,4]. Electrophysiology (e.g. electroencephalography topography, event-related potentials (ERP), and magnetoencephalography (MEG), brain imaging (e.g. magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET) and even newer technologies, such as near-infrared spectroscopy (NIRS) and magnetic resonance spectroscopy (MRS), are versatile tools available to confirm psychogeriatric diagnoses [5]. Furthermore, genetic information is routinely used to evaluate the risk, as well as the prognosis, of a disease and a patient's response to drug treatment [6].

Treatment of behavioral and psychological symptoms of dementia (BPSD) remains one of the most unmet needs in psychogeriatrics [7,8], with more effective pharmacological [9,10] and non-pharmacological interventions [11-13] needed. Psychogeriatrics is, however, a clinical subspecialty in which treatment should be directed towards the person as a whole. Consideration of the person and holistic care are essential, including a bio-

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psycho-socio-ethical evaluation of each patient, because the life of the elderly is so different [1]. Furthermore, psychogeriatric services can be applied to patients in the prestages of dementia, including those with mild cognitive impairment (MCI) [14,15] and subjective cognitive impairment (SCI) [16]. Dementia patients, including those with MCI and SCI, can benefit from psychogeriatric services, and the specific application of laughter and humor therapy in the treatment of these patients is discussed in the present article.

Dementia patients require individualized and life-long intervention

In 2005, it was reported that there were 13.7 million dementia patients in the Asia Pacific region alone and that this number is expected to increase to 64.6 million by the year 2050, a 4.7-fold increase in just 45 years [1]. In addition to its high prevalence, the considerable disruption to patients' daily lives, the burden to caregivers, and the long duration of the disease make dementia, especially Alzheimer's disease (AD), the most malignant disease of our time.

The symptoms of AD differ between individual patients. At the onset of dementia in some patients, certain personality traits that had been well controlled in the past become accentuated, whereas in others there is a 'loss of personality', where the uniqueness of the patient's personality is lost. Some patients show a more rapid deterioration of cognitive function, whereas others show a slower rate of cognitive decline. Some patients exhibit various types of BPSD, whereas others exhibit few abnormal behaviors [7]. Furthermore, the physical, personal, familial, economic, and social environments differ between patients. Thus, each patient should be evaluated as an individual in terms of his/her needs for intervention, taking into account previous social functioning, family structure, and the patient's living environment in order to deliver the most appropriate care. Interventions for dementia patients need to be individualized further taking into consideration the different genetic and environmental factors that are specific to each patient.

The premorbid mental capacity differs between subjects and the symptoms exhibited by dementia patients vary quite widely. Considering the difference in symptoms of dementia patients, a more individualized treatment and management program should be considered taking into account of the emotional and affective responses of each patient individually. In this respect, the possibility of using laughter and humor therapy as a complementary and alternative medicine (CAM) for the treatment of dementia patients is discussed below.

Discussion

Laughter as a CAM

Although modern medical science has enabled correct diagnoses to be made and proper treatments to be initiated for acute diseases caused by exogenous pathogenic factors, there are still numerous chronic, incurable diseases caused by endogenous factors, such as cancer, dementia, hypertension, diabetes, chronic pain etc., for which there is no effective treatment, leaving patients with these conditions to suffer. To facilitate the better management of these chronic diseases, recent attention has focused on the use of CAM, together with Oriental and traditional medicines [17]. CAM is defined by the American Cancer Society as '...supportive methods used to complement evidence-based treatment. Complementary therapies do not replace mainstream treatment and are not promoted to cure disease. Rather, they control symptoms and improve well-being and quality of life'[18]. In contrast, alternative therapies, or alternative medicine, involve non-mainstream treatments that are sometimes used by patients instead of orthodox treatments. Examples of CAM include music therapy, drama therapy, aromatherapy, animal-assisted therapy, gardening, horse riding, exercise, bathing, herbal medications, acupuncture, moxibustion, shiatsu, and yoga among others [19]. However, these therapies have not been well defined. Some are simply based on legend or belief, whereas others are traditionally applied but without any scientific basis.

It is widely accepted that a patient's emotional state will affect the course of the disease. Human emotional behavior can be either negative or positive. Negative emotional behavior is accompanied by disgust, fear, or alarm, which induces a prompt, narrowed response to the stimulus responsible for the life crisis. The 'fight-or-flight' response is the general outcome of negative emotions, in which the sympathetic autonomic nervous system is dominant. Conversely, in safe and relaxing situations, positive emotional behavior is associated with joy, play, and humor, with predominant functioning of the parasympathetic system, which induces responses of extended open behaviors that are helpful in learning new behavioral patterns. Laughter associated with a pleasant feeling is often observed under positive emotional conditions.

Laughter has a unique position in CAM. The benefits of laughter have been recognized historically. As stated by Bertrand Russell, 'Laughter is the most inexpensive and most effective wonder drug. Laughter is a universal medicine'. Laughter has been regarded as beneficial for human health for a long time, with some of the benefits

attributed to laughter including improved immunological [20] and endocrinological [21] responses and increased pain tolerance [22]. Laughter therapy, humor therapy, laughter meditation, and laughter clubs all have unique implications as group programs and as self-management techniques. For practitioners to implement credible programs and effectively teach self-management techniques, further empirical research on the physical, psychosocial, and placebo effects of laughter and humor needs to be conducted.

Physiology of laughter and smiling

Speech and laughter are unique to humans. Although there is considerable information regarding the neuronal representation of speech, little is known about the neural mechanisms of laughter. As described by Charles Darwin, laughter, which is a ubiquitous and unique maneuver of humans that results in a totally defenseless posture involving movement of such a wide area of musculature, should have some beneficial meaning in terms of the evolution of this species [23]. Laughter should mean a lot to our lives.

Newborn babies smile within the first 5 weeks after birth and laugh within the first 4 months. Some smiles are voluntary and smiling can be differentiated into 16 different expressions [24], but there is only one expression of laughter. When we smile, the mouth angles are lifted and the orbits of the eye become thin and surrounded by wrinkles as a result of the simultaneous contraction of the muscularis zygomaticus major and orbicularis oculi. In addition to these muscles, when a person is laughing a wider area of the musculature, including facial, pharyngeal, and respiratory muscles, is simultaneously contracted [24].

Laughter and smiling are usually produced as a message of good will to others. In primates, facial expressions showing bared teeth mean friendliness and primates use these expressions to transmit their sociability and the fact that they have no hostile feelings. Because some forms of smiling are voluntary and easily faked, laughter, which requires a more synergetic contraction of the wider musculature, is believed to have evolved in humans to express a secure, safe message to others.

Neural circuits of laughter and smiling

Laughter is the physiological opposite of crying and is usually an expression of happiness involving typical facial movements and contractions of the respiratory muscles [25]. Neural correlates for laughter may include the anterior cingulate gyrus, which provides emotional consciousness to an individual's experience and is partially under the control of the frontal cortex [26]. The caudal hypothalamus is also involved, acting as the center coordinating emotional changes, including laughter, whereas the temporal amygdala may provide emotional coloring to perceptions and aid in understanding humor [26,27]. Finally, the ventral pontomedullary center for laughter coordinates facial expressions, expirations, and emotional vocalization.

The expression of laughter depends on two partially independent neuronal pathways. One is the 'involuntary' system involving the amygdala, thalamic, hypothalamic and subthalamic areas, and the dorsal brain stem [27]; the other is 'voluntary' and originates in the premotor opercular areas, leading through the motor cortex and the pyramidal tract to the ventral brain stem.

The neural circuit underlying laughter may have three main brain components: (i) cognitive areas, such as sections of the frontal lobe that help a person understand the situation; (ii) a movement area (probably the supplemental motor area) that triggers muscle movements to induce a smile or laughter; and (iii) an emotional component that actuates the perception of happiness after an amusing experience, possibly facilitated by the nucleus accumbens [28].

Neural circuits of humor

Humor can be broadly defined as 'something that is, or is designed to be, comical or amusing'. More specific definitions vary, but humorous communication certainly causes increased feelings of happiness and laughter in those who respond to it, whether due to witty comments or amusing behavior.

Freud's psychodynamic viewpoint described humor as the strongest form of the defense mechanism that allows an individual to face problems and avoid negative emotion [29]. Humor is believed to be effective in distancing oneself, framing problems with perspective, and proactively managing distress [30-32].

Although physiological research on the effects of humor on the body is only just developing, there may be quantifiable health care benefits of humor. Research involving additional measurements of a sense of humor, including self-reported instruments, peer ratings, and comedy monologues, suggests that humor moderates the impact of stressful life events on mood disturbances, such as depression and anxiety, salivary immunoglobulin, and positive affect [33-35]. Similar moderating effects of humor have been identified for depression, insomnia, loneliness, and self-esteem, although not for anxiety [36-39].

Good humor makes people laugh just like pain makes people cry, but humor requires complex neural circuits. Humor is perceived at the beginning as surprise or disharmony, then the paradox is solved, and, finally, the punch line is understood in association with a pleasant feeling. The appreciation of humor requires a wide area of neural circuits covering attention, working memory,

flexible thinking, extraction of word meaning, and positive mood. Patients with lesions in the right frontal lobe have difficulty appreciating humor because of impaired integration of cognition and emotion. Different brain areas are activated by jokes/puns and comics [40]. Humor is present in any social situation, and the nature of what is perceived as amusing varies widely among individuals, societies, and cultures. Everyone enjoys laughing, but a misjudged humorous comment can cause offense, so although laughter is almost always positive, humor itself can provoke mixed emotional responses.

Classification of laughter and smiling

Laughter and smiling can be classified into one of three categories based on evolutional staging as follows: (A) that evoked by a release of tension; (B) that associated with pleasant feelings; and (C) that used for social communication (Table 1).

Laughter or smiling caused by a release of tension is the most basic biological form, and occurs spontaneously in an individual who experiences release from a strenuous tension. The purpose of laughter in this context has been hypothesized to be the release of inner energy accumulated in response to the stress [23]. Laughter to relax is important for the maintenance of mental health. Long-lasting mental tension is accompanied by a hyperaroused state of the sympathetic nervous system, which can be released by laughing [24]. From the viewpoint of mental health, laughter evoked in response to the release of tension is the most important.

The second category, laughter that is provoked or accompanied by pleasant feelings, can be further subdivided into laughter caused by: (B1) fulfillment of instinctive needs; (B2) fulfillment of expectations; (B3) a feeling of superiority; and (B4) recognition of mix-ups. As early as 5 weeks after birth, babies smile after feeding. This is the first laughter observed in human life, elicited by a fulfillment of instinctive needs. Similar laughter is observed in adults after a good meal or a good sleep. When our expectations are realized, especially after hard work and/or endeavor, we usually laugh in association with pleasant feelings, which can be amplified by colleagues sharing in our achievement, with the most explosive form of laughter then being observed. Laughter caused by a feeling of superiority is a type of scornful laughter or a cold smile that has been proposed by some researchers to be the prototype of laughter [23]. Laughter associated with disharmony and/or mismatch is caused by simple mistakes or funny happenings that cause no harm. This sort of laughter can be elicited only when the disharmony is sudden, unexpected, and the results of the misunderstanding are harmless.

Table 1: Relationship between laughter/smiling and the progression of dementia

Type of laughter/smile	Preservation in dementia	
	Early stages	Advanced stages
A1. Release from strong tension	+	+
A2. Release from weak tension	+	+
B1. Fulfillment of instinctive needs	+	+
B2. Fulfillment of expectations	+	-
B3. Feelings of superiority	+	-
B4. Feelings of disharmony	+/-	-
C1. Cooperative	-	-
C2. Defensive	-	-
C3. Aggressive	-	-
C4. Devaluating	-	-

The type of laughter and/or smiling can be classified into one of three categories: (A1,2) that evoked by a release of tension; (B1-4) that associated with pleasant feelings; and (C1-4) that used for social communication. Laughter and smiling induced by a release of tension is regarded as the most basic type and is preserved as the phylogenetically primitive type. Laughter and smiling associated with pleasant feelings has developed with the evolution of humans. Laughter and smiling as communication tools are the most sophisticated and have developed with the sociability of humans. Dementia patients lose the ability to laugh and smile as the disease progresses. Laughter and smiling as communication tools may be lost in the early stages of dementia, when the clinical symptoms of dementia appear. Of the different forms of laughter and smiling associated with pleasant feelings, those induced by disharmony may be lost in early stages of dementia because of the cognitive impairment that may limit a patient's understanding. However, laughter and smiling induced by feelings of superiority, fulfillment of expectations, and fulfillment of instinctive needs are preserved until the advanced stages of dementia. Laughter and smiling in response to a release of tension are preserved in most dementia patients.

The third category of laughter is that used as a communication tool. Facial expressions are important components of laughter and we use these expressions to transmit our intention to be friends with others. Laughing and smiling used to communicate with others can be further subdivided into laughter and smiling for cooperation, defense, aggression, and devaluation. A typical example of cooperative smiling is that used as a greeting. We usually say hello and shake hands while smiling. A defensive smile can be observed when someone is trying to conceal their inner feelings, whereas aggressive laughter can also be called scornful laughter. Everyone dislikes being laughed at and, consequently, aggressive laughter is

extremely powerful. Smiling to devalue something is often used in daily life; for example, when the train door shuts in our face, we often give a wry smile to cancel out the impact of the event.

Laughter in dementia patients

Laughter is usually provoked or accompanied by positive emotions. In clinical settings, it is always desirable for patients, their families, and staff to share relaxed and happy feelings, because patients are often under continuous strain and enormous pressure as a result of their illness. The more serious the illness, the more overwhelming the strain to the patients and their families. Dementia patients are usually under considerable strain, at least at the beginning of their illness. Patients' families are placed under even more stress because of the burden of care [41]. A positive emotion, together with laughter, may enable dementia patients to cope with their illness better, improve immune function, increase pain tolerance, and decrease the stress response. When a positive attitude is shared by patients and staff, it can have a positive effect on the emotional-affective and cognitive functioning of the patients [42,43].

Because the social life of dementia patients is impaired by their illness, they can easily feel isolated. Thus, a feeling that unites them, or provides some sort of bond, with their family and the community can be very beneficial. Dementia patients are often encouraged to participate in daily activities with other people and the positive emotions that are shared by the patients and the care staff help the patients maintain social contact.

Several psychosocial interventions are applied to dementia patients in clinical settings [44]. Examples include cognitive rehabilitation, reminiscence therapy, art therapy, drama therapy, and aerobic exercise [45]. In these activities, a positive attitude of patients is essential and it is always true that a greater effect can be expected when patients participate willingly with a positive outlook. In the case of cognitive rehabilitation, active participation is the condition under which good outcomes can be expected. If the patients are reluctant to participate in the activities, it is unlikely that the program will have any beneficial effects.

Dementia patients become anxious and irritated because they are unable to glean sufficient information from their surroundings due to their impaired cognitive functioning [46]. They are easily trapped in a state in which they feel unsafe, alarmed, and insecure, which, in turn, reduces their ability to process information from their surroundings. With even less secure information, they become more alarmed, leading to negative emotional behavior.

Dementia patients often show various types of BPSD during the course of their illness. Aggression, refusal to cooperate, negativity, and apathy are common, all of which contribute to the further isolation of these patients. In this sense, it is important to keep patients with BPSD within the community.

Because BPSD can often be the most formidable barrier to the care of dementia patients, it is highly recommended that the occurrence of BPSD is prevented. To reduce the occurrence of BPSD in dementia patients, patients should be kept in a stable and safe environment, efforts should be made to ensure good communication with the patients, and patients should be kept feeling relaxed and safe. By doing so, the patients are more likely to laugh and smile.

It is true that laughter and smiling decrease over time in most dementia patients, but it is important to note that not all forms of laughter and smiling are equally reduced. The ability to laugh for social communication is readily lost by dementia patients at the onset of their illness, concomitant with the loss of a social life and their ability to process information, but laughter in response to the release of tension is preserved until the advanced stages of the disease. When dementia patients are released from either physical or mental strain, they always smile. Laughter caused by feelings of disharmony is not usually preserved in dementia patients because of impaired cognitive functioning and because these patients are no longer able to understand the meaning of complicated situations, which means they often cannot understand the punch lines of jokes or appreciate humor.

As discussed above, laughter associated with pleasant feelings can be further subdivided into four types, fulfillment of instinctive needs, fulfillment of expectations, a feeling of superiority, and recognition of mix-ups. Most laughter associated with pleasant feelings is preserved in dementia patients, with observations indicating that these patients laugh and smile when they are exposed to pleasant stimuli. They smile when they are well fed and when they have had a good sleep. They also smile and laugh when they have attained self-set goals. Laughter associated with feelings of superiority is clearly preserved in most dementia patients; they become happy and pleasant when their superiority is recognized. Conversely, when these patients feel humiliated, they become angry and insulted.

Thus, the basic form of laughter is preserved in dementia patients, but the social form of laughter is sometimes lost in the advanced stages of the disease. It is important to ensure that dementia patients are kept in a safe and relaxed environment (and not in alarmed and tensioned),

which will make it more likely that these patients will be able to laugh and smile.

Humor in dementia patients

Humor has positive physiological and psychological effects in a variety of situations. The psychiatric literature purports humor as an effective tool in psychiatric illness and psychotherapy. Benefits of humor in business, management, education, and clinical settings are widely recognized because the right perspective facilitates problem solving both interpersonally and in a group setting. Furthermore, humor puts people at ease, promoting the expression and exchange of ideas. Not only can humor benefit patients, but the use of humor can facilitate the effective management of staff and others in the health care setting [22].

Humor is delicate and sensitive by nature. Humor can be properly appreciated when it is expressed in the right time, right place, and on the right occasion. Confidence, or trust, between the sender and receiver is an important aspect of humor. Establishing this trust is a prerequisite for the introduction of appropriately timed humor. No humor can be appreciated by patients when there is no trust between the patient and care staff. If one side is defensive or angry, he/she may find that the use of humor by the other party is offensive or insulting [47,48]. Patients may also become upset about jokes made at their expense, fearing humiliation and stigmatization [49]. The appropriateness of humor depends on the culture, education, and cognitive function of the receiver. Therefore, the use of humor must be timed wisely and it must be used carefully.

Dementia patients may be more sensitive to jokes or humor than healthy people because patients in the early stages of the disease know that they have difficulties understanding complicated things. Dementia patients with cognitive impairment have difficulty appreciating the disharmony in information sent as humor. Humor should be presented to dementia patients after close evaluation. There are no definitive rules, but humor should generally be introduced slowly; if there is no response or the response is negative, it may be a good idea to abandon all attempts to introduce humor, at least during that clinical encounter [50]. Humor can be used as a defense mechanism in an adverse setting and has obvious value for dementia patients if it is properly addressed and accepted. But the impaired cognitive function of dementia patients must be kept in mind so that humor is presented at the right time, in the right place, and on the right occasion. Everyone enjoys laughing, but a misjudged humorous comment can cause offense, so although laughter is almost always positive, humor itself can provoke mixed emotional responses.

> The other reactions--anger, depression, suppression, denial--took a little piece of me with them. Each made me feel just a little less human. Laughter made me more open to ideas, more inviting to others, and even a little stronger inside. It proved to me that, even as my body was devastated and my spirit challenged, I was still a vital human being. Scott Burton [51]

Summary

Dementia patients should be cared for taking into consideration their individual capacities, which differ from patient to patient. Most laughter and smiling is preserved in dementia patients until the end of the clinical course, even though laughter and smiling as a means of communication is lost during the early stages of the disease. Laughter and smiling associated with pleasant feelings, with the exception of laughing in response to feelings of disharmony, and laughter induced by the release of tension can be used in the treatment of dementia patients. The use of humor, covering issues of the fulfillment of instinctive needs and expectations, as well as feelings of superiority (Table 1), can be a good and effective complementary and alternative intervention in the treatment of dementia patients.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MT, TK, and TT discussed the importance of laughter and humor to dementia patients and drafted the manuscript. MO, ST, and TM searched for the data on the topics in the literatures. MT, RH, and GS devised the table. All authors have read and approved the final manuscript.

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References

1. Takeda M: **Psychogeriatrics in Japan-Bio-Psycho-Socio-Ethical Model.** *Psychogeriatrics* 2001, **1**:83-87.
2. Tanaka T, Tomioka M, Sadik G, Takeda M: **The 13th Congress of the International Psychogeriatric Association and recent expansion of research into psychogeriatrics.** *Psychogeriatrics* 2008, **8**:1-3.
3. Imai K, Yamaguchi K, Watanabe M, Kainuma E, Hikake N, Saitoh S, Tanaka M, Morita M, Sunayama T, Nomura M, Kozakai T, Wada S, Ueki K, Kimura R: **Crucial role of thalami and basal ganglia in emotional memory and cognition: association with the recognition of Niigata Ken Chuetsu earthquake 2004.** *Psychogeriatrics* 2007, **7**:58-63.
4. Yano M, Umeda S, Miura M: **Preserved priming but insensitivity to perceptual fluency on recognition judgements in Alzheimer's disease.** *Psychogeriatrics* 2008, **8**:178-187.
5. Nakajima K, Takahashi M, Oishi S, Inoue A, Sawayama E, Kamiya M, Tanaka S, Miyaoka H: **The relationship between psychiatric symptoms and regional cerebral blood flow in the patients with mild Alzheimer's disease.** *Psychogeriatrics* 2008, **8**:108-113.
6. Kudo T, Tanii H, Takeda M: **Neurodegerative dementias involving aberrant protein aggregation.** *Psychogeriatrics* 2007, **7**:114-117.
7. Hamuro A, Isono H, Sugai Y, Mimura M, Kamijima : **Characteristics of behavioral and psychological symptoms of dementia in untreated oldest old Alzheimer's disease.** *Psychogeriatrics* 2008, **8**:8-11.

8. Kosaka K: **Behavioral and psychological symptoms of dementia (BPSD) in dementia with Lewy bodies.** *Psychogeriatrics* 2008, **8**:134-136.
9. Tanaka T, Kazui H, Morihara H, Sadik G, Kudo T, Takeda M: **Efficacy and safety of donepezil hydrochloride in patients with Alzheimer's disease with behavioral and psychological symptoms of dementia (BPSD).** *Psychogeriatrics* 2008, **8**:114-123.
10. Mizukami K: **Kampo therapy as an alternative to pharmacotherapy using antipsychotic medicines for behavioral and psychological symptoms of dementia (BPSD).** *Psychogeriatrics* 2008, **8**:137-141.
11. Okumura Y, Tanimukai S, Asada T: **The effects of short-term reminiscence therapy on elderly with dementia: A comparison with everyday conversation approaches.** *Psychogeriatrics* 2008, **8**:124-133.
12. Kinoshita T: **The role of home visit medical service to patients with BPSD (behavioral and psychological symptoms of dementia) in community.** *Psychogeriatrics* 2008, **8**:142-147.
13. Takita M: **How to treat BPSD, do not treat patients having symptoms like BPSD with neuroleptics for the first time.** *Psychogeriatrics* 2008, **8**:148-150.
14. Meguro K: **Community based measures form an aging mild cognitive impairment: The Osaki-Tajiri Project.** *Psychogeriatrics* 2007, **7**:132-136.
15. Mimura M, Komatsu S: **Cognitive rehabilitation and cognitive training for mild dementia.** *Psychogeriatrics* 2007, **7**:137-143.
16. Takeda M, Morihara T, Okochi M, Sadik G, Tanaka T: **Mild cognitive impairment and subjective cognitive impairment.** *Psychogeriatrics* 2008, **8**:155-160.
17. **Complementary and Alternative Medicine, When Rigorous, can be Science.** *Evid Based Complement Alternat Med* 2004, **1**:1-4.
18. Ernst E: **The role of complementary and alternative medicine in cancer.** *Lancet Oncol* 2000, **1**:176-180.
19. Kawamura N, Niiyama M, Niiyama H: **Long-term evaluation of animal-assisted therapy for institutionalized elderly people: A preliminary result.** *Psychogeriatrics* 2007, **7**:8-13.
20. Takahashi K, Iwase M, Yamashita K, Tatsumoto Y, Ue H, Kuratsune H, Shimizu A, Takeda M: **The elevation of natural killer cell activity induced by laughter in a crossover designed study.** *Int J Mol Med* 2001, **8**:645-50.
21. Hayashi K, Hayashi T, Iwanaga S, Kawai K, Ishii H, Shoji S, Murakami K: **Laughter lowered the increase in postprandial blood glucose.** *Diabetes Care* 2003, **26**:1651-2.
22. Stuber M, Hilber SD, Mintzer LL, Castaneda M, Glover D, Zeltzer L: **Laughter, humor and pain perception in children; a pilot study.** *Evid Based Complement Alternat Med* 2009, **6**:271-276.
23. Darwin C: **The Expression of the Emotions in Man and Animals.** 3rd edition. Oxford: Oxford University Press; 2002:1-512.
24. Sumitsuji N: **The origin of intermittent exhalation (A! Ha! Ha!) peculiar to human laugh.** *Electromyogr Clin Neurophysiol* 2000, **40**:305-309.
25. Provine R, Yong YL: **Laughter: a stereotyped human vocalization.** *Ethology* 1991, **89**:115-124.
26. Devinsky O, Morrell MJ, Vogt BA: **Contributions of anterior cingulated cortex to behaviour.** *Brain* 1995, **118**:279-306.
27. Wild B, Rodden FA, Grodd W, Ruch W: **Neural correlates of laughter and humour.** *Brain* 2003, **126**:2121-2138.
28. Brain Briefings Ariniello L: **Humor, Laughter and the Brain.** Washington: Society for Neuroscience; 2001.
29. Freud S: **Jokes and their relation to the unconscious.** Edited by: Stanchey J. New York: W. W. Norton and Company; 1960:1-296.
30. Buxman K: **Humor in therapy for the mentally ill.** *J Psychosoc Nurs Ment Health Serv* 1991, **29**:15-18.
31. Groves DF: **"A merry heart doeth good like a medicine..".** *Holist Nurs Pract* 1991, **5**:49-56.
32. Bennett MP, Lengacher C: **Humor and laughter may influence health:Part2. Complementary therapies and humor in a clinical population.** *eCAM* 2006, **3**:187-190.
33. Martin R, Lefcourt HM: **Sense of humor as a moderator of the relation between stressors and moods.** *J Pers Soc Psychol* 1983, **45**:1313-1324.
34. Martin RA, Dobbin JP: **Sense of humor, hassles, and immunoglobulin A: evidence for a stress-moderating effect of humor.** *Int J Psychiatry Med* 1988, **18**:93-105.
35. Martin RA: **Humor, laughter, and physical health: methodological issues and research findings.** *Psychol Bull* 2001, **127**:504-19.
36. Anderson C, Arnoult LH: **An examination of perceived control, humor, irrational beliefs, and positive stress as moderators of the relation between negative stress and health.** *Basic Appl Soc Psych* 1989, **10**:101-117.
37. Nezu AM, Nezu CM, Blissett SE: **Sense of humor as a moderator of the relations between stressful events and psychological distress: a prospective analysis.** *J Pers Soc Psychol* 1988, **54**:520-525.
38. Overholser J: **Sense of humor when coping with life stress.** *Pers Individ Dif* 1992, **13**:799-804.
39. Porterfield A: **Does sense of humor moderate the impact of life stress on psychological and physical well-being?** *J Res Pers* 1987, **21**:306-317.
40. Iwase M, Ouchi Y, Okada H, Yokoyama C, Nobezawa S, Yoshikawa E, Tsukada H, Takeda M, Yamashita K, Takeda M, Yamaguchi K, Kuratsune H, Shimizu A, Watanabe Y: **Neural Substrates of Human Facial Expression of Pleasant Emotion Induced by Comic Films: A PET Study.** *NeuroImage* 2002, **17**:758-768.
41. Rokkaku R: **Support for families with a family member suffering a cognitive disorder.** *Psychogeriatrics* 2007, **7**:144-146.
42. Rosenheim E, Golan G: **Patients reactions to humorous interventions in psychotherapy.** *Am J Psychother* 1986, **40**:110-124.
43. Epstein B: **The use of humor in cognitive-behavioral therapy with outpatient depressed male adolescents.** *Sci Eng* 1997, **57**:5915.
44. Yamamoto-Mitani N, Matsuoka K, Fujii M: **Home-based rehabilitation program for older adults with cognitive impairment: Preliminary results.** *Psychogeriatrics* 2007, **7**:14-20.
45. Yamagami T, Oosawa M, Ito S, Yamaguch H: **Effect of activity reminiscence therapy as brain-activating rehabilitation for elderly people with and without dementia.** *Psychogeriatrics* 2007, **7**:69-75.
46. Savaskan E, Müller SE, Boehringer A, Philippsen C, Mueller-Spahn F, Schaechinger H: **Age determines memory for face identity and expression.** *Psychogeriatrics* 2007, **7**:49-57.
47. Rosenheim E, Golan G: **Patients' reactions to humorous interventions in psychotherapy.** *Am J Psychother* 1986, **40**:110-124.
48. Epstein B: **The use of humor in cognitive-behavioral therapy with outpatient depressed male adolescents.** *Sci Eng* 1997, **57**:5915.
49. Chapple A, Ziebland S: **The role of humor for men with testicular cancer.** *Qual Health Res* 2004, **14**:1123-1139.
50. Joshua AM, Cotroneo A, Clarke S: **Humor and oncology.** *J Clin Oncol* 2005, **23**:645-648.
51. Burton S: **Why Not Laugh?** [http://www.sburton.com/whynotlaugh.htm]. accessed Feb 2009

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A functional polymorphism in the disrupted-in schizophrenia 1 gene is associated with chronic fatigue syndrome

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ABSTRACT

Aims: Disrupted-in schizophrenia 1 (DISC1), identified in a pedigree with a familial psychosis with the chromosome translocation (1:11), is a putative susceptibility gene for psychoses such as schizophrenia and major depressive disorder (MDD). Patients with chronic fatigue syndrome (CFS) report having continuous severe fatigue and many overlapping symptoms with MDD; however, the mechanism and effective treatment of CFS are still unclear. We focused on the overlapping symptoms between CFS and MDD and performed an association study of the functional single-nucleotide polymorphism (SNP) in the DISC1 gene with CFS.

Main methods: Venous blood was drawn from CFS patients and controls and genomic DNA was extracted from the whole blood according to standard procedures. Ser704Cys DISC1 SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay.

Key findings: We found that the Cys704 allele of Ser704Cys SNP was associated with an increased risk of CFS development compared with the Ser704 allele.

Significance: DISC1 Ser704Cys might be a functional variant that affects one of the mechanisms implicated in the biology of CFS. Some patients with CFS showed a phenotype similar to that of patients with MDD, but further studies are needed to clarify the biological mechanism, because this study is of a rather preliminary nature. Despite the variety of patients with CFS, DISC1 Ser704Cys has an association with CFS, which may also suggest that *DISC1* plays a central role in the induction of various psychiatric diseases.

Introduction

The disrupted-in schizophrenia 1 (DISC1) gene was initially identified at the breakpoint of a balanced translocation (1,11)(q42.1;q14.3), which segregated it from major mental disorders in a large Scottish family (Millar et al. 2000). In this family, patients with schizophrenia, bipolar disorder, and recurrent major depressive disorder (MDD) were identified as carriers of the translocation (Millar et al. 2000; Blackwood et al. 2001). Subsequent genetic studies in several independent populations, including association and linkage studies, have also suggested that the DISC1 gene may be implicated in schizophrenia, bipolar disorder, and MDD (Ekelund et al. 2001, 2004; Hennah et al. 2003; Hodgkinson et al. 2004; Sachs et al. 2005; Thomson et al. 2005; Hashimoto et al. 2006). Chronic fatigue syndrome (CFS) is a disorder diagnosed following at least 6 months of disabling, unexplained mental and physical fatigue accompanied by other physical and psychological

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symptoms (Fukuda et al. 1994; Prins et al. 2006). Patients with CFS have an inordinately high rate of depression symptoms or MDD. Clinical samples indicate that between 35% and 46% exhibit depressive symptoms (Johnson et al. 1996; Ciccone et al. 2003) and 31% have MDD (Henderson and Tannock 2005). Among a clinical sample of individuals with CFS, patients concurrently experiencing depressive symptoms were found to have significantly worse outcomes, such as more persistent symptoms and unemployment, than those without depressive symptoms (Bombardier and Buchwald 1995). However, no association study between the DISC1 gene and CFS has been reported, despite the observations that some of the symptoms typically observed in CFS were also common in MDD and that patients with CFS had high rates of major current and lifetime depressive episodes (Afari and Buchwald 2003). Here, we report an association between the functional single-nucleotide polymorphism (SNP) Ser704Cys of DISC1 and CFS.

Materials and methods

Subjects

Subjects for the clinical association study were recruited at Fatigue Clinical Center in Osaka City University Hospital, Osaka, Japan, and the Osaka University Hospital Department of Psychiatry. Enrolled in the study were 155 patients with CFS [55 men and 100 women with a mean age of 36.0 years (SD: 8.51 years)] and 502 healthy control subjects [236 men and 266 women with a mean age of 65.3 years (SD: 19.0 years)]. All of the subjects were Japanese. Of the patients with CFS, 59% also had psychiatric diseases. We classified psychiatric disorders based on ICD-10 (World Health Organization 1992): F3 (Mood Disorders including MDD), $N=7$; F4 (Neurotic, Stress-related and Somatoform Disorder), $N=69$; F3 and F4, $N=3$; F51 (Nonorganic Sleep Disorder), $N=3$; and Unknown, $N=9$. Individual diagnoses were made according to the criteria for CFS of the Centers for Disease Control and Prevention (Fukuda et al. 1994). Control subjects were healthy volunteers who had no current or past connection with psychiatric services. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki and all procedures were carried out with the adequate understanding and written consent of the subjects. The study protocol was approved by institutional ethics committees.

Detection of SNP

Venous blood was drawn from subjects and genomic DNA was extracted from the whole blood according to standard procedures. DISC1 has several SNPs but only the Ser704Cys DISC1 SNP shows a significant association with brain function and it might be a functional variant that affects neural mechanisms (Hashimoto et al. 2006). In addition, robust effects of DISC1 on ERK and Akt signaling and evidence that the Cys704 DISC1 (the risk allele for MDD) might exert a weaker effect on the ERK activation than Ser704 DISC1 have been shown (Hashimoto et al. 2006). One related SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay as described previously (Hashimoto et al. 2005a,b). Primers and probes for detection of the SNPs are available upon request. Statistical analyses were performed using SNPAlyze Pro software, version 5.1.1 (DYNACOM, Yokohama, Japan) and SPSS 16.0 J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were analyzed using χ^2 tests for sex and the *t*-test for age. Genotypic and allelic distributions between patients and controls were analyzed by the χ^2 test. All *P*-values reported are two-tailed. Hardy–Weinberg equilibrium for the SNP (Ser740Cys) in controls and patients was examined to test the genotype distribution. Statistical significance was defined as $P<0.05$.

Results

The genotype distribution was in Hardy–Weinberg equilibrium for the SNP (Ser740Cys) in the group of patients with CFS ($P=0.12$) and controls ($P=0.21$). We examined the association between the genetic variant Ser704Cys SNP and CFS (Table 1). The Cys allele frequency of Ser704Cys was significantly greater in patients with CFS when compared with controls [$\chi^2=4.50$, df$=1$, $P=0.037$, odds ratio$=$ 1.50, 95%CI (confidence interval): 1.12–2.19]. There were no differences among genotype groups (A/A, T/T, and A/T) between CFS patients and the controls ($\chi^2=4.47$, df$=2$, $P=0.10$).

There was no significant difference among genotype groups between male CFS patients and controls ($\chi^2=1.24$, df$=2$, $P=0.54$) but there were significant differences between female CFS patients and controls ($\chi^2=8.81$, df$=2$, $P=0.012$). The Cys allele frequency of Ser704Cys was significantly greater in female patients with CFS when compared with controls ($\chi^2=10.37$, df$=1$, $P=0.0013$, odds ratio$=2.12$, 95% CI: 1.33–3.36) but not in male patients, consistent with previous association study in schizophrenia (Hennah et al. 2003).

Discussion

We initially found evidence for an association between CFS and the functional Ser704Cys SNP in the DISC1 gene. The association level was slightly weak because CFS includes various types of patients, some of whom also have psychiatric diseases or symptoms, such as depressive symptoms and MDD, creating a syndrome. False-positive associations due to population stratification cannot be excluded in our case–control study, despite the precaution of ethnic matching. The mean age in patients with CFS was significantly younger than that of the controls ($t=26.9$, df$=575.4$, $P<0.001$) and the frequency of females in the patients with CFS was significantly higher than that of the controls ($\chi^2=6.38$, df$=1$, $P=0.012$). This is a limitation of the study and differences in sex ratio and ages between groups could be potential confounding factors. Therefore, it is necessary to carry out further investigations to confirm our findings in other samples. The other limitation of the study was that we have not done stratified analysis by looking at CFS only, CFS with MDD, MDD, and healthy controls. Physicians recommend CFS patients who are suspected to be comorbid with psychiatric diseases to visit psychiatrists, however some of them refuse to visit psychiatrists, and thereafter it is difficult to discriminate patients with MDD and other psychiatric disorders exactly. Schrijvers et al. (2009) reported that the relationship between CFS and MDD (and other psychiatric disorders) remains an area of controversy and they mentioned these two reasons. First, the fundamental issue is one of diagnostic labeling for symptom-based disorders in the absence of biological markers or a clear aetiology (Afari and Buchwald 2003). Second, MDD and CFS are heterogeneous conditions. As to this heterogeneity, CFS shares some clinical and

Table 1
Genotype and allele distributions of single-nucleotide polymorphisms in the DISC1 gene between patients with chronic fatigue syndrome and healthy controls.

Marker dbSNP IDs rs821616	Amino acid substitution Ser704Cys	*N*	Genotype (A2110T)			Genotype *P*-value (χ^2) df$=2$	MAF	Allelic *P*-value (χ^2) df$=1$	OR (95%CI)
			A/A	A/T	T/T		*T*		
CFS		155	177	32	6	0.10 (4.47)	**0.14**	**0.037 (4.50)**	**1.50 (1.12–2.19)**
CON		502	410	84	8		**0.10**		

CFS, patients with chronic fatigue syndrome; CON, healthy controls; MAF, minor allele frequency; OR, odds ratio; 95%CI, 95% confidence interval.

neurobiological characteristics with the atypical subtype of MDD (American Psychiatric Association 1994). It is the limitation of clinical research. The study is of a rather preliminary nature, because the difference in the DISC1 Ser740Cys allele frequencies between CFS patients and controls is very small.

DISC1 is a multi-functional protein. Several research groups have identified DISC1-interacting proteins that are associated with the components of the cytoskeleton and centrosomes, such as dynein, Nudel, and elongation protein zeta-1 (Kamiya et al. 2005; Millar et al. 2003; Morris et al. 2003; Miyoshi et al. 2003, 2004; Ozeki et al. 2003). DISC1 plays critical roles in the cerebral cortex development via microtubular dynamics and the DISC1–dynein complex (Kamiya et al. 2005). Another function of DISC1 may be the modulation of cAMP signaling via an interaction with phosphodiesterase 4B, which also has been found to be disrupted by a balanced translocation in a patient with schizophrenia (Millar et al. 2005). Phosphodiesterase inhibitors may have a role in the treatment of certain neuropsychiatric fatigue-related conditions, such as CFS (Staines et al. 2009). Other functions of DISC1, including mitochondrial and nuclear functions, have also been suggested (Morris et al. 2003; Sawamura et al. 2005; James et al. 2004). Mitochondrial ability was significantly associated with CFS severity (Myhill et al. 2009). Hashimoto et al. (2006) revealed that healthy subjects who carried the risk allele for MDD (Cys704 DISC1) had relatively reduced gray matter volumes in their cingulate cortex. Previous studies revealed a reduction in gray matter volume in the bilateral prefrontal cortex in patients with CFS (Okada et al. 2004). CFS shows results that were reportedly generated by DISC1 (Kamiya et al. 2005; Millar et al. 2003, 2005; Morris et al. 2003; Miyoshi et al. 2003, 2004; Ozeki et al. 2003; Staines et al. 2009; Sawamura et al. 2005; James et al. 2004; Myhill et al. 2009). DISC1 Ser704Cys might be a functional variant that affects one of the mechanisms implicated in the biology of CFS. Despite the variety of patients with CFS, DISC1 Ser704Cys has an association with CFS, which may also suggest that *DISC1* plays a central role in the induction of various psychiatric diseases. *DISC1* might be more central to human psychological functioning than previously thought (Tomppo et al. 2009). However, the mechanism or causes of CFS are still unclear. Further studies are needed to clarify the neural mechanism of the Cys allele in patients with CFS.

Conclusion

In summary, the Cys704 allele of Ser704Cys SNP was associated with an increased risk of CFS development compared with the Ser704 allele. DISC1 Ser704Cys might be a functional variant that affects one of the mechanisms implicated in the biology of CFS. Some patients with CFS showed a phenotype similar to that of patients with MDD, but further studies are needed to clarify the mechanism and the cause of CFS, because this study is of a rather preliminary nature.

Conflict of interest statement

We have no conflict of interest to declare in all of the above categories.

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References

Afari N, Buchwald D. Chronic fatigue syndrome: a review. American Journal of Psychiatry 160 (2), 221–236, 2003.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Association, Washington DC, 1994.

Blackwood DH, Fordyce A, Walker MT. St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders — cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. American Journal of Human Genetics 69 (2), 428–433, 2001.

Bombardier CH, Buchwald D. Outcome and prognosis of patients with chronic fatigue vs chronic fatigue syndrome. Archives of Internal Medicine 155 (19), 2105–2110, 1995.

Ciccone DS, Busichio K, Vickroy M, Natelson BH. Psychiatric morbidity in the chronic fatigue syndrome: are patients with personality disorder more physically impaired? Journal of Psychosomatic Research 54 (5), 445–452, 2003.

Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R, Suhonen J, Ellonen P, Chan G, Sinsheimer JS, Sobel E, Juvonen H, Arajärvi R, Partonen T, Suvisaari J, Lönnqvist J, Meyer J, Peltonen L. Chromosome 1 loci in Finnish schizophrenia families. Human Molecular Genetics 10 (15), 1611–1617, 2001.

Ekelund J, Hennah W, Hiekkalinna T, Parker A, Meyer J, Lönnqvist J, Peltonen L. Replication of 1q42 linkage in Finnish schizophrenia pedigrees. Molecular Psychiatry 9 (11), 1037–1041, 2004.

Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Annals of Internal Medicine 121 (12), 953–959, 1994.

Hashimoto R, Okada T, Kato T, Kosuga A, Tatsumi M, Kamijima K, Kunugi H. The breakpoint cluster region gene on chromosome 22q11 is associated with bipolar disorder. Biological Psychiatry 57 (10), 1097–1102, 2005a.

Hashimoto R, Suzuki T, Iwata N, Yamanouchi Y, Kitajima T, Kosuga A, Tatsumi M, Ozaki N, Kamijima K, Kunugi H. Association study of the frizzled-3(FZD3) gene with schizophrenia and mood disorders. Journal of Neural Transmission 112 (2), 303–307, 2005b.

Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, Mori T, Nemoto K, Adachi N, Izumi A, Chiba S, Noguchi H, Suzuki T, Iwata N, Ozaki N, Taguchi T, Kamiya A, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Sawa A, Kunugi H. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology, and ERK signaling. Human Molecular Genetics 15 (20), 3024–3033, 2006.

Henderson M, Tannock C. Use of depression rating scales in chronic fatigue syndrome. Journal of Psychosomatic Research 59 (3), 181–184, 2005.

Hennah W, Varilo T, Kestilä M, Paunio T, Arajärvi R, Haukka J, Parker A, Martin R, Levitzky S, Partonen T, Meyer J, Lönnqvist J, Peltonen L, Ekelund J. Haplotype transmission analysis provides evidence of association for DISC1 to schizophrenia and suggests sex-dependent effects. Human Molecular Genetics 12 (23), 3151–3159, 2003.

Hodgkinson CA, Golman D, Jaeger J, Persaud S, Kane JM, Lipsky RH, Malhotra AK. Disrupted in schizophrenia 1(DISC1): association with schizophrenia, schizoaffective disorder, and bipolar disorder. American Journal of Human Genetics 75 (5), 862–872, 2004.

James R, Adams RR, Christie S, Buchanan SR, Porteous DJ, Millar JK. Disrupted in Schizophrenia 1 (DISC1) is a multicompartmentalized protein that predominantly localizes to mitochondria. Molecular and Cellular Neuroscience 26 (1), 112–122, 2004.

Johnson SK, DeLuca J, Natelson BH. Depression in fatiguing illness: comparing patients with chronic fatigue syndrome, multiple sclerosis and depression. Journal of Affective Disorder 39 (1), 21–30, 1996.

Kamiya A, Kubo K, Tomoda T, Takaki M, Youn R, Ozeki Y, Sawamura N, Park U, Kudo C, Okawa M, Ross CA, Hatten ME, Nakajima K, Sawa A. A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nature Cell Biology 7 (12), 1167–1178, 2005.

Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, Devon RS, Clair DM, Muir WJ, Blackwood DH, Porteous DJ. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Human Molecular Genetics 9 (9), 1415–1423, 2000.

Millar JK, Christie S, Porteous DJ. Yeast two-hybrid screens implicate DISC1 in brain development and function. Biochemical and Biophysical Research Communications 311 (4), 1019–1025, 2003.

Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, Malloy MP, Chubb JE, Huston E, Baillie GS, Thomson PA, Hill EV, Brandon NJ, Rain JC, Camargo LM, Whiting PJ, Houslay MD, Blackwood DH, Muir WJ, Porteous DJ. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science 310 (5751), 1187–1191, 2005.

Miyoshi K, Honda A, Baba K, Taniguchi M, Oono K, Fujita T, Kuroda S, Katayama T, Tohyama M. Disrupted-In-Schizophrenia 1, a candidate gene for schizophrenia, participates in neurite outgrowth. Molecular Psychiatry 8 (7), 685–694, 2003.

Miyoshi K, Asanuma M, Miyazaki I, Diaz-Corrales FJ, Katayama T, Tohyama M, Ogawa N. DISC1 localizes to the centrosome by binding to kendrin. Biochemical and Biophysical Research Communications 317 (4), 1195–1199, 2004.

Morris JA, Kandpal G, Ma L, Austin CP. DISC1 (Disrupted-in-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5, and NUDEL: regulation and loss of interaction with mutation. Human Molecular Genetics 12 (13), 1591–1608, 2003.

Myhill S, Booth NE, McLaren-Howard J. Chronic fatigue syndrome and mitochondrial dysfunction. International Journal of Clinical Experimental Medicine 2 (1), 1–16, 2009.

Okada T, Tanaka M, Kuratsune H, Watanabe Y, Sadato N. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. BMC Neurology 4 (1), 14, 2004.

Ozeki Y, Tomoda T, Kleiderlein J, Kamiya A, Bord L, Fujii K, Okawa M, Yamada N, Hatten ME, Snyder SH, Ross CA, Sawa A. Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. Proceedings of the National Academy of Sciences USA 100 (1), 289–294, 2003.

Prins JB, van der Meer JW, Bleijenberg G. Chronic fatigue syndrome. Lancet 367 (9507), 346–355, 2006.
Sachs NA, Sawa A, Holmes SE, Ross CA, DeLisi LE, Margolis RL. A frameshift mutation in Disrupted in Schizophrenia 1 in an American family with schizophrenia and schizoaffective disorder. Molecular Psychiatry 10 (8), 758–764, 2005.
Sawamura N, Sawamura-Yamamoto T, Ozeki Y, Ross CA, Sawa A. A form of DISC1 enriched in nucleus: altered subcellular distribution in orbitofrontal cortex in psychosis and substance/alcohol abuse. Proceedings of the National Academy of Sciences USA 102 (4), 1187–1192, 2005.
Schrijvers D, Hulstijn W, Sabbe BGC. Psychomotor functioning in chronic fatigue syndrome and major depressive disorder: a comparative study. Journal of Affective Disorders 115 (1–2), 46–53, 2009.
Staines DR, Brenu EW, Marshall-Gradisnik S. Postulated vasoactive neuropeptide immunopathology affecting the blood–brain/blood–spinal barrier in certain neuropsychiatric fatigue-related conditions: a role for phosphodiesterase inhibitors in treatment? Neuropsychiatric Disease and Treatment 5, 81–89, 2009.
Thomson PA, Wray NR, Millar JK, Evans KL, Hellard SL, Condie A, Muir WJ, Blackwood DH, Porteous DJ. Association between the TRAX/DISC locus and both bipolar disorder and schizophrenia in the Scottish population. Molecular Psychiatry 10 (7), 657–668, 2005.
Tomppo L, Hennah W, Miettunen J, Järvelin MR, Veijola J, Ripatti S, Lahermo P, Lichtermann D, Peltonen L, Ekelund J. Association of variants in DISC1 with psychosis-related traits in a large population cohort. Archives of General Psychiatry 66 (2), 134–141, 2009.
World Health Organization. ICD-10: The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines. World Health Organization, 1992.