Table 1
Demographic information and COMT Val/Met genotype

Variables	Val/Val ($n=77$)	Val/Met ($n=45$)	Met/Met ($n=17$)
Age	37.2 (12.9)	35.9 (12.3)	33.2 (5.9)
Gender (M/F)	29/48	13/32	5/12
Education years	16.6 (2.9)	16.2 (2.2)	17.4 (4.1)
IQ	110.8 (11.5)	110.3 (11.8)	107.0 (15.2)
WCST PE	4.3 (5.1)	3.7 (4.1)	3.5 (3.7)

Mean ± S.D. There was no significant difference among genotypes for any variable. WCST PE: Wisconsin Card Sorting Test Preservative Errors.

and the Wisconsin Card Sorting Test [10,13] were administered to all subjects. Subjects with significant medical problems, history of head of trauma, neurosurgery and alcohol or substance abuse were excluded. They had no current or past contact with psychiatric services. All subjects were biologically unrelated Japanese. After a description of the study, written informed consent was obtained from every subject. The study protocol was approved by an institutional ethical committee. Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The subjects were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, as described previously [18]. Primers and probes for detection of the SNP were: forward primer 5′-GACTGTGCCGCCATCAC-3′; reverse primer 5′-CAGGCATGCACACCTTGTC-3′; probe 1 5′-VIC-TTTCGCTGGCGTGAAG-MGB-3′; and probe 2 5′-FAM-CGCTGGCATGAAGMGB-3′. PCR cycling conditions were: at 95 °C for 10 min, 50 cycles of 92 °C for 15 s and 60 °C for 1 min. Statistical tests were carried out using SPSS for Windows version 11.0 (SPSS Japan, Tokyo) and a power analysis program (R version 2.5.1: http://www.r-project.org/index.html). Group comparisons of demographic data were performed by using analysis of variance (ANOVA) or χ^2, as appropriate. The effects of the COMT genotype on scales of TCI, IQ or WCST were assessed by ANOVA or multiple regression. A Spearman rank order correlation test was used for comparisons between HA score and age, gender and education. Statistical significance was defined as $p<0.05$. As the statistical considerations are essential to carry out the association study between genetic polymorphisms and personality [12,17], we further applied correction of multiple comparisons and power analyses to avoid type 1 and 2 errors.

We examined the effects of the COMT genotype on the following measures of the TCI in a cohort of 139 normal subjects: novelty seeking (NS), harm avoidance (HA), reward dependence (RD), persistence (P), self-directedness (SD), cooperativeness (C) and self-transcendence (ST). Table 1 gives the means and standard deviations of age, education years, full scale of IQ, and preservative errors of the WCST and gender distribution for groups defined by COMT Val/Val ($n=77$), Val/Met ($n=45$) and Met/Met ($n=17$) genotypes. There were no differences among genotype groups and demographic variables or tasks of general academic ability, for example, IQ or preservative errors in the WCST (Table 1). An ANOVA detected a significant effect of genotype on HA ($F=4.08$, df=2,136, $P=0.019$) (Fig. 1), but no significant difference was observed among genotype

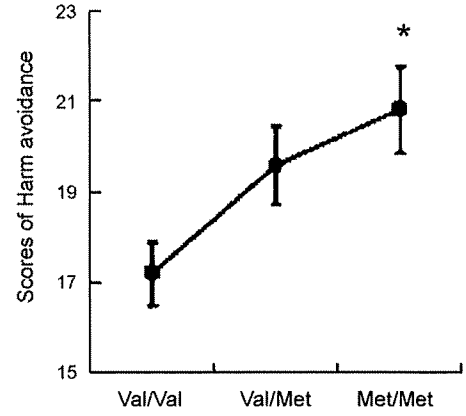

Fig. 1. Harm avoidance and COMT Val/Met genotype. Scores of harm avoidance in healthy individuals with Val/Val ($n=77$), Val/Met ($n=45$) and Met/Met ($n=17$) genotypes are shown (mean ± S.D.). *$P<0.05$, significant difference compared with Val/Val.

groups in NS, RD, P, SD, C or ST (Table 2). Post hoc comparisons showed that Val/Val homozygote subjects had lower scores than Met/Met ($p=0.013$) and Val/Met ($p=0.09$) subjects. Prior reports have shown the effect of age on the HA score [3]. When we examined the effect of age, gender or education years on the HA score, we found that age was negatively correlated with HA (Rho = −0.378, $p<0.00001$), whereas gender and education years were not (gender: Rho = 0.073, $p=0.39$, education years: Rho = −0.048, $p=0.57$). Multiple regression with the number of Met alleles and age as continuous factors revealed both factors to be parametrically related HA scores ($r^2=0.22$, $F=19.9$, $p<0.00001$, Met allele: $t=2.5$, $P=0.013$; age: $t=-5.5$, $P<0.00001$). Thus, the number of Met alleles was positively correlated with a higher HA score in our sample.

There is a risk of type I errors occurring: some comparisons that are statistically significant at a 5% level may be because of chance. When multiple comparison was applied in the effect of genotype on HA score, 0.71% level is appropriate. Thus, the COMT genotype effect on HA in the Japanese population (ANOVA: $p=0.019$, Multiple regression: $p=0.013$) should be considered to be a trend level. Replication studies in Japanese populations are necessary to confirm the present results.

Kim et al. [14] reported a significant association between the Val/Met polymorphism and harm avoidance only in female

Table 2
COMT Val/Met genotype and Temperament and Character Inventory (TCI)

Scales	Val/Val ($n=77$)	Val/Met ($n=45$)	Met/Met ($n=17$)
NS (novelty seeking)	21.5 (3.9)	22.1 (5.0)	20.5 (4.3)
HA (harm avoidance)	17.2 (6.2)	19.6 (5.7)	20.8 (4.0)*
RD (reward dependence)	15.1 (3.9)	15.7 (3.8)	14.8 (2.9)
P (persistence)	4.4 (2.0)	4.7 (1.7)	4.6 (2.0)
SD (self-directedness)	30.1 (6.4)	28.7 (5.5)	29.8 (5.0)
C (cooperativeness)	29.0 (4.4)	28.3 (5.6)	29.8 (4.1)
ST (self-transcendence)	10.3 (4.7)	11.2 (4.6)	9.8 (4.6)

Mean ± S.D. Significantly different compared with Val/Val (*$P<0.05$).

subjects in a Korean population, which is a close ethnicity to Japanese. Their results indicated a higher HA score in subjects with a Val/Val genotype. These data are opposite to our results, showing a higher HA score in subjects with a Met/Met genotype. Thus, we examined the association between the Val/Met SNP of the COMT gene and HA in male and female subjects separately. Individuals of both sexes with a Met/Met genotype showed higher HA scores than Val/Val or Val/Met individuals (male: Val/Val 16.4, Val/Met 19.6, Met/Met 20.6; female: Val/Val 17.7, Val/Met 19.6, Met/Met 20.9); however, this difference did not reach statistical significance (male: $p = 0.06$, female: $p = 0.13$). As our sample size is small, a power analysis was performed to evaluate the statistical power. The power of our sample to detect average differences of HA scores between genotypes in female subjects was calculated using a one-tailed alpha value of 0.05. Our sample size had a power (0.8) to detect average differences between Val/Val and Val/Met: 3.4, Val/Val and Met/Met: 4.6, and Val/Met and Met Met: 4.2. As the average differences of HA score between genotypes in the Korean study were 3.3 Val/Val and Val/Met), 5.8 (Val/Val and Met/Met), and 2.5 (Val/Met and Met Met), our sample size had 0.8 of power at least in the comparison between Val/Val and Met/Met.

There were several studies to investigate the relationship between the COMT Val/Met polymorphism and personality traits in other ethnic populations. Benjamin et al. [1,2] showed that the COMT Val/Met polymorphism and interaction of the COMT genotype and 5-HTTLPR were associated with persistence (RD2) and NS. Subsequently, Tsai et al. [22] reported that the COMT Val/Met genotype was associated with NS and RD but not with HA and Lichtenberg et al. [15] failed to find an association between the COMT Val/Met genotype and personality traits. We could not replicate the association between the COMT genotype and other personality traits such as NS, P in other ethnicity. These inconsistencies may relate to sample differences, false positive results, false negative results and possible genetic and allelic heterogeneity. In addition, other genetic factors such as the polymorphisms of the 5HTT, DRD2, DRD3, and DRD4 genes, environmental factors, and gene and environment interactions might influence personality traits [17]. Replication studies using a larger sample size and/or Japanese, Korean or Caucasian cohorts would be required to draw any conclusion.

In this study, we reported a possible association between the Val/Met polymorphism of the COMT gene and HA assessed by the TCI in a Japanese population. The number of Met alleles was positively correlated with a higher HA score. The Met-type COMT protein has lower catecholamine metabolism activity than the Val-type protein, which might lead to a hyper-dopaminergic state and higher activity in the prefrontal cortex. Exposure of rodents to stressful stimuli increased cortical dopamine, while diazepam, an anxiolytic benzodiazepine, could reverse the effect of dopamine increase by stressful stimuli [11], indicating that hyper-dopaminergic transmission in frontal cortex might induce anxious state. Thus, higher activity in the prefrontal cortex could explain the higher HA score and higher anxiety in normal subjects.

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Prepulse inhibition of acoustic startle in Japanese patients with chronic schizophrenia

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Abstract

Prepulse inhibition (PPI) of acoustic startle reflex has been suggested as a neurophysiologic measure of information processing abnormalities in schizophrenia. However, there has been little information on PPI and related measures in Asian patients with schizophrenia. We examined startle response to acoustic stimuli, its habituation, and PPI in 20 Japanese patients with chronic schizophrenia under antipsychotic medication and 16 healthy controls matched for age and sex. We measured PPI with 115 dB of pulse (40 ms), 82, 86, or 90 dB of prepulse (20 ms) and 30, 60, or 120 ms of lead interval (LI). The startle response to pulse alone trials was significantly smaller in schizophrenics than in controls, which may be due, at least in part, to medication. There was no significant difference in habituation of startle response during the test session between the two groups. PPI differed significantly between the two groups when LI was 120 ms. No significant relationship was found on startle response or PPI with age of onset, number of previous admission, medication dosages, or symptom scores assessed with the Positive and Negative Syndrome Scale (PANSS). Our results confirm impaired PPI in chronic schizophrenia patients compared with controls in Japanese.

Keywords: Acoustic startle response; Prepulse inhibition; Habituation; Schizophrenia; Japanese; Parameters

1. Introduction

Prepulse inhibition (PPI) of acoustic startle reflex has been suggested as a neurophysiologic measure of information processing abnormalities in schizophrenia (reviewed by Cadenhead and Braff, 1999; Braff et al., 2001a,b). This deficit of PPI may reflect a biological correlate of sensory flooding and cognitive fragmentation in individuals with schizophrenia. Furthermore, PPI shows substantial heritability (Anokhin et al., 2003), and it has been considered to be a reliable intermediate phenotype of sensorimotor gating deficits in schizophrenia that could be useful in genetic studies as well as diagnostic tests (Braff and Light, 2005). However, PPI is substantially dependent on measurement parameters such as sound pressure of prepulse and lead interval (LI) between pulse and prepulse (Blumenthal, 1999; Braff et al., 2001a,b). Moreover, a recent study has suggested ethnic differences in startle magnitude and PPI between Caucasian and Asian subjects (Swerdlow et al., 2005), indicating the possible importance of determining optimal test parameters in Asian subjects. To our knowledge, however, there has been little information on PPI and related measures from Asian populations, and no published data have been thus far available on whether PPI is impaired in Asian patients with schizophrenia.

The aims of the study were to examine startle response to acoustic stimuli, its habituation during the test session, and PPI

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in Japanese patients with chronic schizophrenia in comparison with healthy controls. We attempted to determine optimal parameters such as sound pressure of prepulse and LI in our sample. Furthermore, we examined the possible relationships of the deficits in PPI with clinical characteristics of the patients.

2. Subjects and methods

2.1. Subjects

Subjects were 20 patients with schizophrenia who were under treatment at the National Center of Neurology and Psychiatry Musashi Hospital, Tokyo, Japan. Consensus diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders 4th ed. (DSM-IV; American Psychiatric Association, 1994) was made by at least two psychiatrists for each patient based on detailed interviews and medical records. All the patients were clinically stable on a stable dose of antipsychotic medication for at least 3 months prior to PPI test. Symptom severity of the patients was assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) by a single psychiatrist (H.K.) at the time of PPI test. Sixteen healthy volunteers served as controls. They were asked if they have had been to psychiatric or psychosomatic medicine clinic for any mental health problem. Individuals who had a current or past history of contact to such clinics or those who had a family history of psychosis (i.e., presence of individuals with current or past contact to psychiatric services for schizophrenia spectrum disorders, depressive disorder or bipolar disorder within the first degree relatives) were not enrolled in the study. The absence of current or past history of major psychiatric illnesses was further confirmed by using the structured interview of the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Otsubo et al., 2005; Sheehan et al., 1998). All the subjects had no difficulty in hearing, which was confirmed by an interview. Age and sex distributions were not significantly different between the patients and controls. All the patients and controls were biologically unrelated Japanese who resided in the same geographical area (western part of Tokyo metropolitan area). After description of the study, written informed consent was obtained from every subject. The study protocol was reviewed and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Measurement of startle response and its prepulse inhibition

Startle reflex to acoustic stimuli was measured by using the Startle Reflex Test Unit for Humans (O'Hara Medical Co., Tokyo, Japan). The room for the measurement was completely sound-proofed and electrically shielded. Subjects refrained from smoking for at least 20 min prior to testing. They were seated comfortably in a couch. They were instructed to be awake and stare at a fixed point. Small electrodes (6 mm in diameter) with gel paste ("Gelaid", Nihon Kohden, Tokyo, Japan) were placed below both eyes over the orbicularis oculi muscle after polishing and cleaning skin surface with skin preparation gel for bioelectrical measurement ("skinPure" by Nihon Kohden, Tokyo, Japan) and 70% isopropyl alcohol for disinfection, and ground electrodes were placed behind ears over the mastoid. Broadband white noise (50–24,000 Hz) of 70 dB was presented as the background noise which was continuously presented afterwards throughout a session. Acoustic startle stimuli of the broadband white noise were presented through headphones. During the initial 5 min of each session, the background noise alone was given for acclimation. In total, 72 trials of startle response were carried out in a session. These trials consisted of three blocks. In the first block, startle response to pulse (sound pressure: 115 dB; duration: 40 ms) alone was recorded for six times. In the second block, startle response to the same pulse with or without prepulse (sound pressure: 82, 86, or 90 dB; duration: 20 ms; LI [onset to onset]: 30, 60, or 120 ms) was measured six times for each condition. The differential conditions of trials were presented in a pseudo-random order; however, the order was the same for all the subjects. In the final block, startle response to pulse alone was again measured for six times (to see habituation of response to pulse alone). Inter-trial intervals (15 s on average, range 10–20 s) were randomly changed. The entire session lasted approximately 30 min. The eye-blink component was measured using electromyographic (EMG) records. The system recorded 1052 epochs of EMG for 600 ms starting 200 ms prior to the onset of prepulse or pulse (for pulse alone trials). EMG activity was low (250 Hz) and high (90 Hz) pass filtered. Startle response was quantified as the peak of EMG waves, observed during 20–120 ms after the onset of pulse stimulus, which were rectified and smoothed by software using a moving average method with a time constant of 10 ms. All recordings were screened to exclude spontaneous eye-blink that was observed immediately before the acoustic stimuli. Eye-blinks observed in EMG during the period 200 ms before the index prepulse/pulse to 20 ms after the onset of pulse were considered to be spontaneous eye-blinks.

We obtained measures of (1) startle response to pulse alone trials in the first block, (2) habituation (%) of startle response during the session calculated by the formula ([1 − mean startle magnitude in block 3/mean startle magnitude in block 1] × 100), and (3) PPI (%) under the formula ([1 − mean startle response with prepulse trials)/mean startle response to pulse alone trials in the second block] × 100).

2.3. Statistical analysis

All the statistical analyses were performed with the SPSS ver11 (SPSS Japan, Tokyo, Japan). *t*-Test and chi-square tests (Fisher's exact test when appropriate) were used to compare means and categorical proportions, respectively. PPI measures with differential parameters were examined with ANOVA with repeated measures on trial parameters. Pearson's correlation was employed to see possible correlation between PPI and clinical characteristics. All *p*-values reported are two-tailed. Statistical significance was considered when *p*-value was < 0.05.

3. Results

Clinical and demographic characteristics of the subjects are presented in Table 1.

3.1. Startle response and habituation

Startle responses in the first block are illustrated in Fig. 1. Mean startle magnitude was significantly reduced in patients than in controls for both left ($t = -2.5$, d.f. = 34, $p = 0.019$) and right ($t = -3.7$, d.f. = 34, $p = 0.001$) sides (Fig. 1). We defined *a priori* the non-responders to the startle stimuli as the smallest 20 percentile in the total subjects; their average value of left and right startle magnitude was <0.05 (digital unit). Five patients and two controls were non-responders. There was no significant difference in any of the clinical characteristics listed in Table 1 between the 5 non-responders and 15 responders in the patient group. Analyses for habituation and PPI were performed in the responders (15 patients and 14 controls).

With respect to habituation of startle response, there was no significant difference between patients and controls for either left ($70.0 \pm 23.0\%$ in patients and $65.9 \pm 19.7\%$ in controls; $t = 0.5$, d.f. = 27, $p = 0.61$) or right ($64.2 \pm 27.4\%$ in patients and $65.4 \pm 22.5\%$ in controls; $t = -0.1$, d.f. = 27, $p = 0.90$) side.

3.2. Prepulse inhibition

PPI (%) measured in nine conditions (three sound pressures by three LIs) in patients and controls are presented in Fig. 2 (data on right PPI are not shown because left and right PPI were essentially similar). Right PPI measures of one patient were not well recorded for unknown reasons and thus excluded from the analysis. We examined the possible effects of side, LI, prepulse

Table 1
Characteristics of the study subjects (mean ± S.D.)

	Patients	Controls	Significance
Number of subjects	20	16	
Male/female	12/8	9/7	$\chi^2 = 0.1$, d.f. = 1, $p = 0.82$
Age (years) mean (range)	42 ± 9 (22–55)	41 ± 13 (20–72)	$t = 0.4$, d.f. = 34, $p = 0.72$
Current smoker/non-smoker	7/13	4/12	$\chi^2 = 0.4$, d.f. = 1, $p = 0.52$
Handedness right/left	20/0	15/1	$p = 0.44$[a]
Out-/inpatients	17/3	–	
Age of onset (years)	21 ± 6	–	
Number of hospitalization	2.0 ± 1.8		
Family history positive/negative[b]	5/15		
Number of medicated patients (%)		–	
Antipsychotics	20 (100%)		
Antiparkinsonian drugs	17 (85%)		
Anxiolytics/hypnotics	15 (75%)		
Medication dosage (mg/day)			
Antipsychotics[c]	852 ± 654		
Antiparkinsonian drugs[d]	3.0 ± 1.9		
Anxiolytics/hypnotics[e]	6.4 ± 5.4	–	
PANSS			
Total score	64.5 ± 16.0	–	
Positive syndrome	13.2 ± 7.6	–	
Negative syndrome	21.8 ± 7.1	–	
General psychopathology	29.6 ± 7.7	–	

[a] Fisher's exact probability.
[b] Positive family history: at least one relative with schizophrenia within the second degree relatives.
[c] Equivalent to chlorpromazine.
[d] Equivalent to biperiden.
[e] Equivalent to diazepam.

intensity (within-subjects factors), sex, smoking, and case–control status (between-subjects factors) on PPI, controlling for age as a covariate by using ANOVA with repeated measures on trial parameters. There was a highly significant effect of LI on PPI ($F = 6.6$, d.f. = 2, 40, $p = 0.003$); however, there was no significant effect of side ($F = 0.3$, d.f. = 1, 20, $p = 0.58$) or prepulse intensity ($F = 0.7$, d.f. = 2, 40, $p = 0.50$). In addition, there was a significant interaction between LI and case–control status ($F = 3.5$, d.f. = 2, 40, $p = 0.039$), suggesting significant differences in PPI between cases and controls depending on LI. No other significant interaction was detected. As shown in Fig. 2, PPI markedly differed depending on parameters, particularly on LI. When the LI was 30 ms, both patients and controls showed augmented startle response (i.e., facilitation), resulting in PPI values of both sides below zero. Although controls showed more facilitation than patients, any of differences did not reach statistical significance. Only when sound pressure was 82 dB, left response showed a statistical trend towards greater facilitation in controls than in patients. When the LI was 60 ms, there was no significant difference in PPI at any sound pressure of prepulse between patients and controls. When the LI was 120 ms, in contrast, all differences reached or approached statistical significance. When the sound pressure of prepulse was 90 dB, highly significant differences in PPI were observed for both left ($t = -2.8$, d.f. = 27, $p = 0.009$) and right ($t = -3.0$, d.f. = 26, $p = 0.006$) sides between patients and controls.

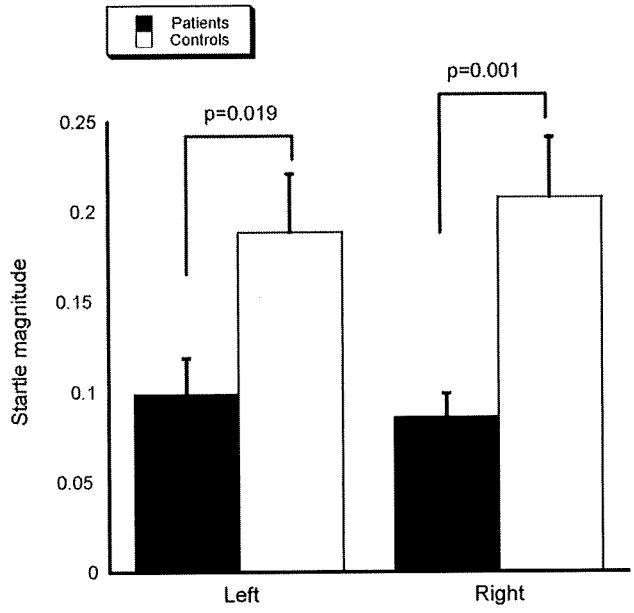

Fig. 1. Startle magnitude in patients and controls.

3.3. Relationship of startle response and PPI with demographic and clinical variables

We examined whether startle response in the first block and PPI had any relationship with demographic and clinical variables within the patients, excluding the non-responders. As

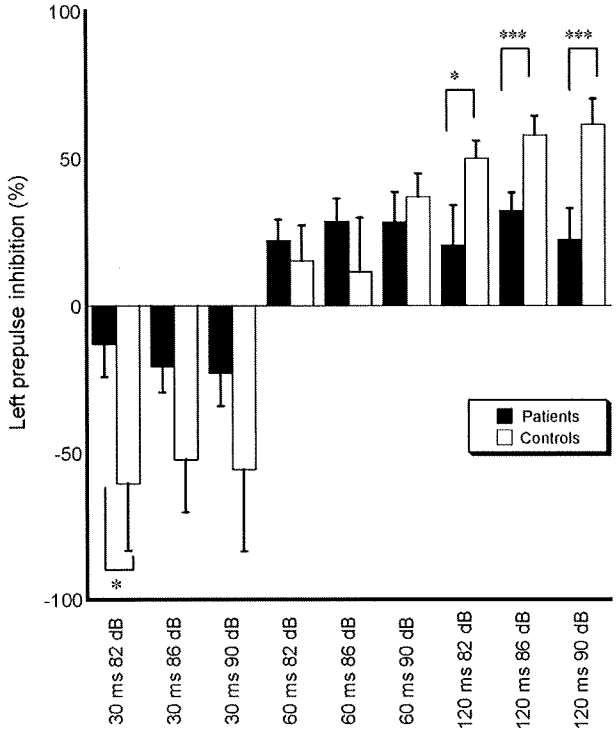

Fig. 2. Prepulse inhibition (PPI) in patients and controls. Records from the left side are shown. Error bars represent standard errors. $*p < 0.10$, $***p < 0.01$.

described above, the greatest statistical differences in PPI between patients and controls were obtained when LI was 120 ms and prepulse 90 dB. Thus PPI values with these parameters were used in the analysis. There was no significant correlation of startle response or PPI for either side with age, age of onset, number of previous admission, any of medication dosages, or any of PANSS scores.

4. Discussion

To our knowledge, the present study is the first report on PPI in Asian (Japanese) patients with schizophrenia. Our main findings were reduced startle response in the initial pulse alone trials and decreased PPI under certain conditions of test parameters in patients with schizophrenia, compared with controls. We found significant differences in PPI between the two groups when LI was 120 ms, but not 30 or 60 ms. With respect to habituation of startle response, no significant difference was found between the two groups in our sample. No significant relationship between clinical variables and PPI was detected in our patients. Although we recorded both left and right sides, there was no substantial difference between the sides for any measure.

4.1. Startle response

We observed substantially reduced startle response in patients with schizophrenia than in controls. In contrast to our finding, the majority of previous studies did not report such a difference in startle response in pulse alone trial (Braff et al., 1978, 1992, 1999, 2001a,b, 2005; Cadenhead et al., 2000; E.J. Duncan et al., 2003; E. Duncan et al., 2003; Ford et al., 1999; Geyer and Braff, 1982; Grillon et al., 1992; Kumari et al., 2002, 2004, 2005a,b; Leumann et al., 2002; Ludewig et al., 2002, 2003; Ludewig and Vollenweider, 2002; Mackeprang et al., 2002; Parwani et al., 2000; Perry et al., 2001, 2004; Swerdlow et al., 2006; Weike et al., 1999; Wynn et al., 2004), although some studies reported a significantly reduced startle magnitude in patients with schizophrenia (Quednow et al., 2006; Meincke et al., 2004). As pointed out by Meincke et al. (2004), it is likely that the observed lower startle response in our patients was attributable, at least in part, to medication. The majority of our patients (75%) received anxiolytics and/or hypnotics, most of which were benzodiazepines. This high rate of prescribing benzodiazepines is not unique to our patients; a relatively high proportion of patients with schizophrenia are co-prescribed with benzodiazepines in Japan, compared with other countries (Bitter et al., 2003). Benzodiazepines have been shown to reduce startle magnitude (Schachinger et al., 1999; Rodriguez-Fornells et al., 1999; Abduljawad et al., 2001). Although we failed to find a significant correlation between daily doses of such drugs (equivalent to diazepam) and startle response (data not shown), this failure is not surprising, given that the patients received differential drugs with differential effects and metabolism rate, and that time lag between drug intake and measurement of startle was not controlled for. Recently, Quednow et al. (2006) also found markedly and significantly reduced startle response in their patients with schizophrenia, compared with controls, at both pre- and post-treatment periods with antipsychotics of amisulpride or olanzapine. In their study, some benzodiazepines were allowed for adjunctive treatment; however, these substances were discontinued 24 h before measurement of startle response, indicating that there is a possibility that reduced startle response occurs in patients with schizophrenia even when effects of benzodiazepines are minimal. Quednow et al. (2006) stated that reduced startle response reflects the "hyporeactivity" in schizophrenia. Further studies controlling for medication status are required to draw any conclusion as to whether startle response at pulse alone trial is altered in schizophrenia.

4.2. Habituation

We failed to find a significant difference in habituation between the patients and controls, which is in line with the majority of previous studies (Braff et al., 2001a,b; Cadenhead et al., 2000; Kumari et al., 2002, 2004, 2005a,b; Leumann et al., 2002; Ludewig et al., 2002; Ludewig and Vollenweider, 2002; Mackeprang et al., 2002; Oranje et al., 2002; Perry et al., 2001, 2004; Swerdlow et al., 2006; Wynn et al., 2004). However, some other studies found reduced habituation in schizophrenia (Braff et al., 1992; Geyer and Braff, 1982; Ludewig et al., 2003; Parwani et al., 2000). Since the majority of the previous studies did not find altered habituation in schizophrenia, the difference in habituation between schizophrenics and controls might be, if any, small, and our sample size might have been too small to detect such a small difference (i.e., type II error).

4.3. Prepulse inhibition

PPI differed markedly depending on LI and intensity of prepulse. When LI was 30 ms, facilitation rather than inhibition of startle response was observed for both patients and controls with no significant difference between the two groups. The facilitated response was likely to result from summation of prepulse and pulse stimuli because of the very short LI. However, the majority of previous studies did not report such facilitated response even when LI was 30 ms (Braff et al., 1978, 1992, 2005; Cadenhead et al., 2000; E.J. Duncan et al., 2003; E. Duncan et al., 2003; Kumari et al., 1999, 2000, 2004, 2005a; Ludewig et al., 2002, 2003; Ludewig and Vollenweider, 2002; Leumann et al., 2002; Mackeprang et al., 2002; Meincke et al., 2004; Parwani et al., 2000; Perry et al., 2001, 2002, 2004; Swerdlow et al., 2005, 2006), although a few studies reported facilitated response only in patients with schizophrenia treated with typical antipsychotics (Kumari et al., 2002, 2005b). The discrepancy between previous studies and ours may be due to ethnic difference; however, this possibility was not supported by Swerdlow et al. (2005) who examined PPI in Asian healthy subjects. Thus there may be some unknown differences in the test procedures between previous studies and ours. We used the Startle Reflex Test Unit for Humans (O'Hara Medical Co., Tokyo, Japan) for recording startle responses. Although this apparatus has been made to be essentially similar to previously used "standard apparatuses (e.g., EMG-SR-LAB; San Diego Instruments, San Diego, California)", there may be some unknown differences between the former and the latter. To elucidate such differences, it is necessary to compare results obtained by the two apparatuses in the same subjects in the same test procedure.

We could not detect any difference in PPI between the patients and controls when LI was 30 or 60 ms; however, we found significant differences in PPI when LI was 120 ms. When LI was 120 ms and prepulse 90 dB, PPI values were highest and the difference between the patients and controls became most significant for both left and right sides, suggesting that the best condition for PPI among the examined conditions might be 120 ms of LI and 90 dB of prepulse in order to discriminate patients and controls in our sample. In the literature, LI values that could discriminate schizophrenics and controls differ across studies. Consistent with our result, many studies reported significantly lower PPI in schizophrenics or a subpopulation of schizophrenics compared with controls when LI was 120 ms (Braff et al., 1992, 2001a,b, 2005; Kumari et al., 1999, 2000; Mackeprang et al., 2002; Oranje et al., 2002; Parwani et al., 2000; Perry et al., 2001, 2002, 2004; Quednow et al., 2006; Weike et al., 1999), while others did not find significant differences (Braff et al., 1978, 1999; Cadenhead et al., 2000; E.J. Duncan et al., 2003; E. Duncan et al., 2003; Grillon et al., 1992; Kumari et al., 2002; Ludewig et al., 2002, 2003; Leumann et al., 2002; Swerdlow et al., 2006; Wynn et al., 2004). In several studies, 60 ms of LI was superior to 120 ms to detect differences between schizophrenia patients and controls (Braff et al., 1978; Kumari et al., 2002; Ludewig et al., 2002, 2003; Leumann et al., 2002; Swerdlow et al., 2006), while in other studies 120 ms was superior to 60 ms (Braff et al., 2005; Parwani et al., 2000). To our knowledge, there was only one study (Cadenhead et al., 2000) that reported 30 ms of LI was superior to other LI values. Taken together, although our results were in favor of 120 ms of LI to discriminate schizophrenics and controls, 60 ms of LI should also be used in the test session.

PPI has been reported to be associated with several clinical characteristics such as severity of positive (Braff et al., 1999; Weike et al., 1999) or negative (Braff et al., 1999) symptoms, thought disorder (Meincke et al., 2004; Perry and Braff, 1994), and age of onset (Kumari et al., 2000). In our sample, however, we could not detect any significant correlation between PPI and clinical variables. Since the present sample size was relatively small, further studies in a larger sample may be necessary to detect such relationships.

5. Conclusions

Our results suggest that startle response in the pulse alone trial was reduced in Japanese patients with schizophrenia compared with controls that may be due, at least in part, to medications of the patients. We confirmed that PPI was reduced in Japanese patients with chronic schizophrenia under stable medication when LI between pulse and prepulse was 120 ms. No apparent relationship was found between PPI and clinical characteristics.

Acknowledgements

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POSTSYNAPTIC DENSITY: A KEY CONVERGENT SITE FOR SCHIZOPHRENIA SUSCEPTIBILITY FACTORS AND POSSIBLE TARGET FOR DRUG DEVELOPMENT

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CONTENTS

Summary

Many studies have supported roles for both genetic and environmental factors in the etiology of schizophrenia. A major hypothesis at present is that schizophrenia is a polygenic disorder where alterations in a set of genes lead to impaired neurodevelopment, which in turn results in altered neurotransmission. Several neurotransmitters, including glutamate, dopamine, serotonin and gamma-amino butyric acid (GABA), have been implicated in schizophrenia, and, as such, there is a growing interest in trying to elucidate the mechanisms whereby alterations in the function of schizophrenia susceptibility gene products can lead to disturbance in signaling at synapses. In this article, we will summarize what is known about schizophrenia susceptibility factors that reside at postsynaptic density (PSD), a unique postsynaptic site where signals from neurotransmitters converge. PSD may be a promising target for novel classes of drugs to treat schizophrenia.

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Introduction

Schizophrenia is a complex neuropsychiatric condition affecting about 1% of the population worldwide. Its etiology remains unclear, although genetic and epidemiological studies suggest that the interplay between genetic and environmental factors during the early stages of neurodevelopment could contribute to its pathogenesis (1, 2). Recently, systematic linkage and association studies have provided a list of susceptibility genes that include *neuregulin (NRG)-1, dysbindin, disrupted-in-schizophrenia 1 (DISC1), neuronal nitric oxide synthase (nNOS)* and *carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON)* (3–5) (Table I).

Brain imaging studies of patients and neuropathological approaches have revealed that schizophrenia patients present with enlarged lateral ventricles, decreased brain volumes and altered synaptic morphology including decreased dendritic spines and arborizations (3, 6–12). These neuroanatomical disturbances have led some to postulate that impaired neurodevelopment may lead to altered neurotransmission in schizophrenia. Interestingly, the majority of genetic susceptibility factors for schizophrenia localize to postsynaptic density (PSD), a unique postsynaptic site, where they may converge and modulate signals downstream of various neurotransmitter receptors (Fig. 1). Of all the neurotransmitters thought to contribute to the pathophysiology of schizophrenia, glutamate has received the most attention. This is because numerous studies have demonstrated that hypoglutamatergic signaling is associated with psychosis. Some studies have found that phencyclidine, a *N*-methyl-*D*-aspartate (NMDA) receptor antagonist, induces schizophrenia-like behaviors in humans (13). In addition, studies in mice, rats and monkeys treated with phencyclidine have shown that these animals displayed behavioral and/or neuroanatomical deficits that resemble those seen in schizophrenia patients (14–16). Thus, PSD linked to NMDA receptors may be a site of convergence for the disease pathways, and, as such, it may be an important target for new drugs to treat schizophrenia. Once we would have identified signaling pathways altered in schizophrenia and their phenotypic outcomes, we can design agents to alleviate or treat these phenotypic insults.

In this review, we will focus on genetic susceptibility factors for schizophrenia that may play a role at PSD associated with NMDA receptors, and discuss potential therapeutic interventions at this site. Our discussion will be centered on the function of these gene products as well as genetic studies linking alterations in these genes to abnormal phenotypes in humans.

Neuregulin-1 and ErbB4

Function

NRG-1 belongs to the family of proteins containing an epidermal growth factor–like motif that is known to activate ErbB1–4 receptors tyrosine ki-

Table I: Susceptibility genes for schizophrenia.

Gene	Chromosomal locus	Function
CAPON	1q22	Regulates the coupling of nNOS to the NMDA receptor via PSD-95
DISC1	1q42	Neurites outgrowth
		Neuronal migration
		Axonal elongation
		Modulates cyclic AMP pathway
Dysbindin	6p22.3	Modulates dopamine release
		Stimulates glutamate release
		Upregulates presynaptic proteins
		Neurotrophic effect through Akt signaling pathway
NRG-1	8p22	Neuronal migration and differentiation
		Modulates $GABA_A$ receptor expression
		Recruits CHRNA7 to synapses
		Regulates expression and plasticity of NMDA receptor
nNOS	12q24	Second messenger of NMDA receptor
		Modulates dopamine and serotonin neurotransmission

NMDA = *N*-methyl-*D*-aspartate; Akt = protein kinase B; GABA = gamma-amino butyric acid.

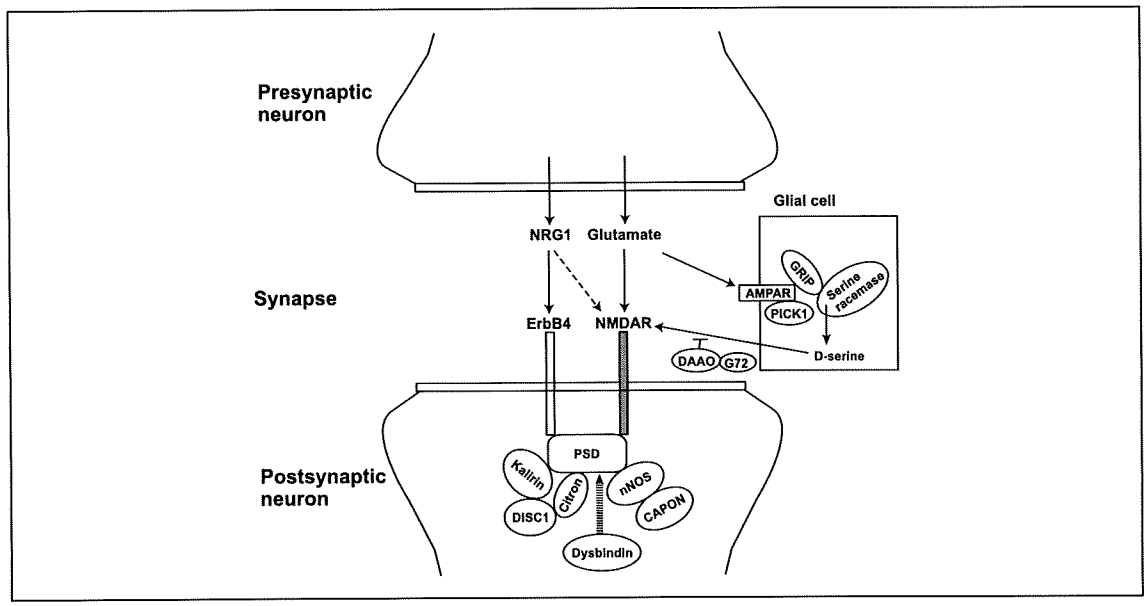

Fig. 1. Schematic representation of schizophrenia gene products localizing at postsynaptic density (PSD). Neuregulin (NRG)-1, dysbindin, disrupted-in-schizophrenia 1 (DISC1), neuronal nitric oxide synthase (nNOS) and carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON) are schizophrenia susceptibility gene products that reside at PSD associated with *N*-methyl-*D*-aspartate receptor (NMDAR). GRIP = glutamate receptor-interacting protein; AMPAR = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor; PICK1 = protein interacting with C kinase 1.

nase (17, 18). NRG-1 has nine known splicing variants that are all expressed in human brain (19). The NRG-1 receptor, ErbB4, has been well characterized. Of most importance, ErbB4 occurs in PSD where it interacts with PSD95, an anchoring protein of the NMDA receptor (20, 21). ErbB4 has several isoforms that are expressed in the brain (22). Splicing variants containing exon 16 encode for ErbB4 isoforms carrying a metalloprotease-sensitive extracellular domain referred to as JM-a. Splicing variants containing exon 26 encode for a cytoplasmic domain containing a phosphotidylinositol-3 kinase (PI3K) binding site referred to as CYT-1.

NRG-1 regulates the expression of three receptors that have been implicated in the pathophysiology of schizophrenia: NMDA, gamma-amino butyric acid (GABA) and nicotinic acetylcholine receptors (23–26). Studies using cerebellar slice cultures have shown that a NRG-beta isoform stimulates the expression of NR2C, an NMDA receptor subunit (27). As discussed below, studies with human tissue have demonstrated a novel function of NRG-1–ErbB4 interaction.

Human studies

Ten independent groups found a genetic association between the *NRG-1* gene and schizophrenia (28–37). A recent study reported for the first time that an NRG-1 variant in individuals at high risk for schizophrenia is associated with impaired activation of frontal and temporal lobes, lower intelligence quotient and increased susceptibility for psychotic symptoms (38).

A study using autopsied human brains reported that type I NRG-1 mRNA is increased in the dorsolateral prefrontal cortex of schizophrenia patients (39). Similar results were obtained in the hippocampus of a much larger and separate sample of schizophrenia patients (40).

Studies using postmortem brains from schizophrenia patients also reported a decrease in the number of oligodendrocytes (41–45). Consistent with these reports, microarray studies have also reported a general reduction in the expression of oligodendrocyte- and myelinization-associated genes in schizophrenia patients (46–48). Since NRG-1 plays an important role in oligodendrocytes development, NRG-1 dysfunction could lead to poor myelination and thus impaired synaptic for-

mation due to improper oligodendrocyte development.

Even though numerous genetic studies in schizophrenia patients have found that the *NRG-1* gene is associated with schizophrenia, we still do not know how alteration in NRG-1 function can lead to the disorder. A study by Hahn *et al.* (49) using postmortem brains from schizophrenia patients suggests that upregulation of NRG-1–ErbB4 signaling suppresses NMDA receptor activation more in schizophrenia patients than in healthy controls. Other studies supporting an association between enhanced NRG-1–ErbB4 signaling and schizophrenia include a report showing a genetic interaction between the Icelandic *NRG-1* haplotype and ErbB4 (37). Another study reported that two ErbB4 isoforms, JM-a and CYT-1, were overexpressed in the dorsolateral prefrontal cortex of schizophrenia patients from their Ashkenazi cohort (50). CYT-1 is a known activator of the PI3K–protein kinase B (Akt) pathway that has been implicated in some cases of schizophrenia.

DISC1

Function

The *DISC1* gene was originally identified at the breakpoint on chromosome 1 resulting from a balanced chromosomal translocation between chromosomes 1 and 11 in a large Scottish family with hereditary psychiatric disorders, including schizophrenia (51, 52). DISC1 is multifunctional and has several subcellular distributions (53). In proliferating cells and developing neurons, a pool of DISC1 forms a complex with dynein, nuclear distribution element-like, and lissencephaly 1 protein at the centrosome (54). Disruption of this complex following suppression of DISC1 expression by RNA interference leads to improper neuronal migration and abnormal dendritic arborizations in the cerebral cortex *in vivo* (54). DISC1 may also function to modulate the cyclic AMP (cAMP) pathway by binding to phosphodiesterase 4B, which itself was found to be disrupted by a balanced chromosomal translocation in two patients with schizophrenia (55). The cAMP pathway is known to modulate learning, memory and mood, and, as such, the interaction between DISC1 and PDE4B suggests that DISC1 may play a role in synaptic plasticity (56–58).

In adult brains, DISC1 turns out to be enriched in PSD and the nucleus. A study demonstrated by immunoelectron microscopy that DISC1 was present in about 8% of axon terminals in the frontal and parietal cortex of healthy subjects (59) (Fig. 2). Furthermore, yeast two-hybrid studies have shown that DISC1 interacts with numerous PSD proteins, including Citron (60, 61).

Human studies

Association of DISC1 with schizophrenia in general has also been supported by various genetic studies (53, 62). For example, a *DISC1* haplotype was found to be undertransmitted in a North American Caucasian population with schizoaffective disorder (63). The same study also reported that haplotype blocks located within exons 1 and 9 are associated with various neuropsychiatric disorders, including schizophrenia, and that the Phe607 allele is overtransmitted in patients with schizoaffective disorders. Another study showed that the Ser704 DISC1 allele is overtransmitted among schizophrenia patients, and that in healthy individuals this allele was associated with defective hippocampal formation and function (64). In addition, several studies have shown that the Ser704Cys DISC1 polymorphism is associated with impaired cognitive function (64–66).

Another study reported that an asymptomatic carrier of the Scottish mutation presented with a deficit in P300 event-related potential, which indicates impairment in higher processes such as memory and attention (67).

Deficits in spatial and working memory function as well as reduced gray matter volume in the dor-

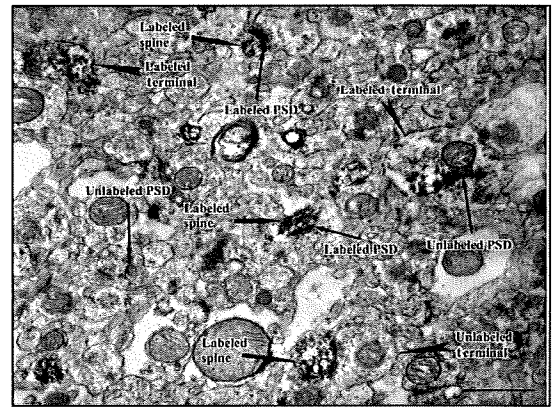

Fig. 2. Electron micrograph showing DISC1 immunoreactivity in various postsynaptic densities (PSD). Reproduced from ref. 59 with permission from John Wiley & Sons, Inc. © 2006.

solateral prefrontal cortex were found in individuals who carry the Finnish *DISC1* risk haplotype, HEP1 (68). Another *DISC1* haplotype, HEP3, has been associated with impaired visual memory and attention (69).

In postmortem brains, we reported that disturbance in the nuclear pool of DISC1 is observed in schizophrenia patients as well as in alcohol/substance abuse patients (70).

Dysbindin

Function

Dysbindin was originally found as a binding partner of alpha- and beta-dystrobrevin, which are causative genes of Duchenne muscular dystrophy (71). Dystrobrevins exist in the dystrophin-associated protein complex that is important for proper muscle function (72). Lack of dystrophin leads to alterations in neuronal membranes that in turn can cause cognitive deficits in affected individuals (73).

Little is known about the functions of dysbindin in neurons. It has been shown that suppression of dysbindin expression in PC12 cells resulted in an increase of dopamine release (74). Another study showed that dysbindin might influence exocytotic glutamate release via upregulation of the presynaptic machinery molecules (75). The same study also reported that dysbindin promotes neuronal viability through PI3K–Akt signaling. A recent study demonstrated that dysbindin interacts with snapin, and the two proteins colocalize in presynaptic sites rich in synaptic vesicle membrane as well as in PSD (76). More studies are needed to elucidate a possible role for dysbindin at PSD.

Human studies

Numerous studies have reported an association between genetic variants of dysbindin and schizophrenia in several populations (75, 77–83).

Some studies have shown that dysbindin single-nucleotide polymorphisms (SNPs) are associated with impairments in general cognitive ability, frontal brain function, as well as more severe negative symptoms in healthy subjects and patients with schizophrenia (84–86). For instance, a study conducted on 213 patients with schizophrenia or schizoaffective disorder found that dysbindin genetic variants that have been associated with schizophrenia can affect intelligence (87). Another study found an association between a dysbindin risk haplotype and spatial working memory (88).

Studies with human autopsied brains have revealed alterations in levels of dysbindin mRNA and protein. One study reported that dysbindin expression is reduced in the hippocampus of schizophrenia patients (89). This reduction was more prominent in the inner molecular layer of the dentate gyrus and correlated with an increase in vesicular glutamate transporter-1. These results suggest that altered glutamatergic signaling may lead to improper hippocampal formation. Another study found decreased dysbindin mRNA levels in the prefrontal cortex and midbrain of schizophrenia patients (90).

nNOS and CAPON

Function

The neuronal form of nitric oxide synthase, also known as neuronal nicotinamide-adenine dinucleotide phosphate-diaphorase (NADPH-d), is the major source of nitric oxide in the central nervous system (91, 92). Nitric oxide is known to function as a second messenger downstream of the NMDA receptor (93). Signal downstream of the NMDA receptor is mediated in part by nNOS, since calcium entering through NMDA receptor channels binds to the calmodulin–nNOS protein complex. CAPON, a new risk factor for schizophrenia, is an adapter protein mediating the coupling of nNOS to the NMDA receptor via PSD95 protein (94).

Human studies

nNOS gene polymorphism has been associated with schizophrenia (95–97). A study showed that the alternative NOS1 exons 1c and 1f are associated with psychosis and altered prefrontal cortex function (96). Another study conducted on 215 Japanese patients with schizophrenia and 182 healthy subjects found an association between an SNP located in exon 29 of the *nNOS* gene and schizophrenia (97). However, another study reported that they did not find an association between a CA dinucleotide repeat polymorphism in the 3'-UTR exon 29 of the nNOS gene and schizophrenia in a Chinese cohort (98).

A study reported various alterations in the morphology as well as the number of NOS-positive neurons in the striatum of schizophrenia patients as measured by immunohistochemistry (99). Another study reported that schizophrenia patients have a reduced number of NADPH-d–positive neurons in the hippocampal formation and neocortex of the lateral temporal lobe (100). This study al-

so found that in schizophrenia patients, the number of NADPH-d–positive neurons was increased in the white matter of the lateral temporal lobe as well as in parahippocampal white matter. There has also been a report of altered distribution of NADPH-d–positive neurons in the frontal lobe of schizophrenia patients (101).

One study reported that patients with schizophrenia and those with bipolar disorder have increased expression of CAPON in the dorsolateral prefrontal cortex (102). According to the authors of this study, one of the consequences associated with increased expression of CAPON is NMDA receptor hypofunction due to disturbance of the interaction between nNOS and NMDA receptor.

Conclusions

NRG-1, DISC1, dysbindin, nNOS and CAPON are examples of key schizophrenia susceptibility factors that could modulate glutamatergic signaling at PSD. Alteration in these factors may mediate the disease-associated disturbance of NMDA–glutamatergic signaling. Thus, agents that can modulate signaling involving these factors at PSD to enhance overall glutamate signaling could potentially be efficacious drugs to treat schizophrenia. Findings from human genetic studies have allowed scientists to make genetically engineered mice displaying schizophrenia-like symptoms, which can be used to elucidate the mechanisms whereby schizophrenia susceptibility genes contribute to its pathophysiology as well as to screen novel classes of drugs for the treatment of schizophrenia.

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The Dysbindin Gene (*DTNBP1*) Is Associated with Methamphetamine Psychosis

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Background: The dysbindin (*DTNBP1* [dystrobrevin-binding protein 1]) gene has repeatedly been shown to be associated with schizophrenia across diverse populations. One study also showed that risk haplotypes were shared with a bipolar disorder subgroup with psychotic episodes, but not with all cases. *DTNBP1* may confer susceptibility to psychotic symptoms in various psychiatric disorders besides schizophrenia.

Methods: Methamphetamine psychosis, the psychotic symptoms of which are close to those observed in schizophrenia, was investigated through a case (n = 197)–control (n = 243) association analyses of *DTNBP1*.

Results: *DTNBP1* showed significant associations with methamphetamine psychosis at polymorphisms of P1635 (rs3213207, $p = .00003$) and SNPA (rs2619538, $p = .049$) and the three-locus haplotype of P1655 (rs2619539)-P1635-SNPA (permutation $p = .0005$). The C-A-A haplotype, which was identical to the protective haplotype previously reported for schizophrenia and psychotic bipolar disorders, was a protective factor ($p = .0013$, odds ratio [OR] = .62, 95% confidence interval [CI] .51–.77) for methamphetamine psychosis. The C-G-T haplotype was a risk for methamphetamine psychosis ($p = .0012$, OR = 14.9, 95% CI 3.5–64.2).

Conclusions: Our genetic evidence suggests that *DTNBP1* is involved in psychotic liability not only for schizophrenia but also for other psychotic disorders, including substance-induced psychosis.

Key Words: Akt1, *DTNBP1*, dysbindin, methamphetamine psychosis, substance dependence

A genetic variation of the dystrobrevin-binding protein 1 (*DTNBP1*) gene has recently been shown to be associated with schizophrenia in several independent studies. Straub *et al.* (1) revealed original evidence for a positive genetic association between schizophrenia and variants in a gene on 6p22.3, dysbindin (*DTNBP1*), which is located within one of several promising loci revealed by a genomewide linkage scan. Many replication studies showed consistent findings in different populations, for example, German (2), Irish (3), Chinese (4), Swedish/German/Polish (5), UK/Irish (5), Bulgarian (6), American (7), Scottish/Chinese (8), and Japanese (9), although the significantly associated alleles and haplotypes were not always consistent among populations. Two postmortem studies also revealed that dysbindin protein or its mRNA level was reduced in the dorsolateral prefrontal cortex and in presynaptic glutamatergic terminals of the hippocampus of schizophrenia patients (10,11). These findings suggest that the dysbindin is involved in the pathogenesis of schizophrenia.

Recently, Raybould *et al.* (12) examined three loci of the *DTNBP1* gene in a large sample of patients with bipolar disorder, another endogenous psychosis, in UK Caucasians, and found that the *DTNBP1* gene was not associated with all cases of bipolar disorder but was associated with a subgroup of bipolar disorder characterized by the complication of psychotic features during episodes. The risk and protective haplotype were identical to those found in their previous schizophrenia study (13). Therefore, they speculated that the *DTNBP1* genetic variation influences susceptibility to schizophrenia and bipolar psychosis across the Kraepelinian dichotomy.

Abuse of large amounts of methamphetamine for long periods easily produces psychotic symptoms, such as delusions of reference, persecution, and poisoning, as well as auditory and visual hallucinations (14–16). Further consumption of methamphetamine may result in severe psychosis, liability to relapse with reconsumption of methamphetamine or psychological stress, and a gradually worsening prognosis. Clinical similarities between methamphetamine psychosis and schizophrenia in a cross-section of clinical features have been noted; these include auditory hallucination and delusion, the longitudinal process of progressive exacerbation with acute relapses, relatively good response to neuroleptics, and enduring vulnerability to relapse to stressors, especially in the paranoid type of schizophrenia. Indeed, methamphetamine psychosis has long been considered a pharmacologic model of schizophrenia (17,18), and shared molecular mechanisms could be involved in these psychotic disorders. Based on this rationale, it is possible that the *DTNBP1*

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