(−30.0, −60.0, 15.0), t=4.47} demonstrated a significant negative correlation with age (Fig. 3). Even if the analysis was done on voxels with FA values higher than 0.35, to examine more anisotropic WM areas, the results were essentially unchanged (data not shown).

3.1.3. Correlational analysis between FA values and clinical factors in schizophrenics

There was a significant negative correlation between FA values and duration of illness in widespread WM areas (Fig. 4), while there was no significant correlation of FA values with age of onset, duration of hospitalization or daily dose of antipsychotic drugs (data not shown).

3.2. ROI analyses

3.2.1. ROI-based correlational analysis in both schizophrenics and controls

First, we constituted a General Linear Model putting diagnosis as a fixed factor and age, IQ and relative WM volume as covariates. F values (significance probabilities) were as follows; diagnosis: 10.8 (P=0.001), age: 26.1 (P<0.001), IQ: 0.029 (P=0.865) and relative WM volume: 16.6 (P<0.001). Then, we added diagnosis-by-age interaction into the model. F values (significance probabilities) changed as follows; diagnosis: 2.34 (P=0.130), age: 27.8 (P<0.001), IQ: 0.059 (P=0.809), relative WM volume: 14.1(P<0.001) and diagnosis-by-age interaction: 7.08 (P=0.009). Effect of IQ was not significant in both models. There was a significant diagnosis-by-age interaction effect.

3.2.2. ROI-based correlational analysis in controls

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with age, IQ and relative WM volume in controls were as follows; FA vs. age: −0.287 (P=0.065), FA vs. IQ: −0.108 (P=0.496) and FA vs. mean WM volume: 0.481 (P=0.001). Only positive correlation between mean WM FA value and relative WM volume was statistically significant.

3.2.3. ROI-based correlational analysis in schizophrenics

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with clinical factors in schizophrenics were as follows; FA vs. age: −0.702 (P< 0.001), FA vs. duration of illness: −0.603 (P<0.001), FA vs. age of onset: −0.305 (P=0.049), FA vs. total daily dose of antipsychotics: 0.110 (P=0.489), FA vs. duration of hospitalization: −0.172 (P=0.277), FA vs. IQ: −0.064 (P=0.686), FA vs. relative WM volume: 0.421 (P=0.006). Significant positive correlation was observed between mean WM FA value and relative WM volume. Fig. 5 shows a scatter plot between age and mean WM FA value in controls and schizophrenics. Fig. 6 shows a scatter plot between duration of illness and mean WM FA value in schizophrenics. Significant negative correlations were observed between mean WM FA value and age (or duration of illness).

4. Discussion

In this study, we obtained three main findings; 1) lower FA values in schizophrenic patients compared with controls in WM areas including frontal and temporal WM, bilateral uncinate fasciculi (external capsules) and cingulum bundles and genu and splenium of corpus callosum, 2) age-related reductions of FA value in the widespread WM were more prominent in schizophrenics than in controls, and 3) a negative correlation between FA value in the widespread WM and duration of illness in schizophrenics.

Recent studies demonstrated age-related FA decline in normal individuals occurred in the prefrontal WM, while temporal WM were relatively preserved (Pfefferbaum et al., 2005; Salat et al., 2005). However, in this study, negative age-dependent effects were observed only in the lenient statistical threshold in the FA values of restricted areas of the WM in controls. This could be explained by the fact that all our subjects were under the age of 60, relatively less old compared to the participants of normal aging studies.

We replicated the results of the most of the previous studies, decreased FA values in the WM of schizophrenics. In the earlier studies concerning FA values in WM of patients with schizophrenia, an inherent abnormality in WM was expected to be detected since the decrease of FA values in the WM of the schizophrenic brain was assumed to occur as neurodevelopmental impairments before onset of the illness. Several studies demonstrated that schizophrenics had reduced FA value in the prefrontal WM (Buchsbaum et al., 1998), prefrontal and parieto-occipital WM (Lim et al., 1999), splenium of the corpus callosum (Agartz et al., 2001) and adjacent occipital WM (forceps major) (Agartz et al., 2001), left uncinate fasciculus and bilateral arcuate fasciculus (Burns et al., 2003), bilateral cingulum bundles (Kubicki et al., 2003). Some of them indicated that the reduction of FA values in schizophrenics might occur independently of reduction of the white matter volume. Although some studies reported no significant FA changes in schizophrenics (Steel et al., 2001; Foong et al., 2002), most studies with chronic

schizophrenia demonstrated lower FA values in schizophrenia (Kanaan et al., 2005). A few DTI studies have examined first episode patients (Price et al., 2005; Szeszko et al., 2005). Szeszko et al. found FA decrease in the left internal capsule and left-hemisphere WM of the middle frontal gyrus and posterior superior temporal gyrus of first-episode schizophrenics and schizoaffective disorder patients, however, the decrease was less pronounced compared with results of the majority of the studies in chronic schizophrenics. On the other hand, Price et al. reported that there was no FA decrease in the corpus callosum of patients with first-episode schizophrenia. They suggested that FA reduction in schizophrenia might reflect neuropathological abnormalities, which may occur after the onset of the disease and could be progressive. Our results, 1) age related FA reduction was more prominent in schizophrenics than controls, and 2) duration of illness was related to FA reduction in schizophrenics, suggest that changes of FA value in schizophrenia are attributable, at least in part, to progressive neuropathological changes after onset of the illness.

Contrary to our results, a previous DTI study demonstrated 'positive' correlation between age and FA in schizophrenics (Jones et al., 2006). They measured FA values of WM tracts captured from tractography, and they set seedpoints of the tracts manually from one slice of FA images. Such methods might overlook general decline of FA values in the WM. Their mean FA values (average of 8 WM tracts in each subjects) were around 0.4, which was relatively higher than those of our study {our mean FA value of entire WM was 0.35+0.01 (mean+S.D.)}. To simulate the analysis of the previous study, we additionally performed an analysis setting masking threshold for FA values of 0.35. As a result, the significant negative correlation remained to be present even in more anisotropic WM areas.

Previous pathological studies demonstrated microscopic abnormalities of the WM in schizophrenia such as decreased expression of myelin and oligodendrocyte-related genes, the decrease in density of oligodendrocytes (Hof et al., 2002), damage of myelin sheath lamellae (Uranova et al., 2001) and maldistribution of interstitial neurons (Akbarian et al., 1996) in prefrontal WM of the brains of schizophrenic patients. Further, a previous longitudinal MR study demonstrated progressive atrophy of the white matter in schizophrenics (Ho et al., 2003). Given these previous findings and ours, it seems likely that age-dependent FA decrease, but not increase, occurs in schizophrenic brains.

As well as a negative correlation with age, FA values of schizophrenics showed negative correlation with duration of illness but not with age of onset or daily dose of antipsychotics. The facts seem to support the hypothesis that FA reduction in schizophrenia might be associated with neuropathological abnormalities which may emerge, at least in part, after the onset of the disease and could be progressive. Further, the spatial distribution of age-related FA reduction in schizophrenics was different from those of normal individuals in previous studies that demonstrated preserved temporal white matter (Pfefferbaum et al., 2005; Salat et al., 2005). Such different distributions suggest that FA changes in schizophrenics might be associated with disease progression rather than merely exaggerated aging effects. However, it is difficult for neuroimaging studies, even for longitudinal studies, to discriminate disease progression from aging effects. The correlational study between DTI findings and pathological findings should be conducted to clarify whether reduction of FA values in schizophrenics reflect pure disease progression or merely exaggerated aging effects.

Several limitations should be considered in our study. First, our study is a cross-sectional study. To confirm progressive pathological process in the WM of the patients of schizophrenia, longitudinal studies should be conducted. Second, IQ score was not matched between groups, i.e., mean IQ score was significantly lower in schizophrenics in our samples. O'Sullivan et al. (2004) reported DTI measures were correlated strongly with cognitive decline in elderly. Thus, it could be problematic whether age-related FA decrease in our study was reflected by cognitive decline. However, no significant correlation was observed between mean WM FA values and IQ in our sample. Also, regarding schizophrenia, it has been hypothesized that most cognitive change takes place early in their psychotic episodes and it remains relatively stable through long term in the illness (Hoff et al., 2005). Hence, at least from our data, we cannot attribute age-related FA decline in schizophrenia to IQ changes. Third, the issue of partial volume effect should be addressed. In schizophrenia, progressive WM atrophy has been reported in the previous studies (Ho et al., 2003). Due to the atrophy, it is possible that the voxels located in the border of the WM and other tissues in schizophrenics were estimated as having lower FA values. However, we minimized the problem by using the high dimensional warping algorithm, threshold masking for FA values and adopting relative WM volume as a nuisance variable. Another issue is the possible effect of long-term medication with antipsychotics. Although daily dose of antipsychotics was not correlated with FA values in schizophrenics, we could not estimate accurate cumulative doses of antipsychotics

throughout the duration of illness. Several morphological MR studies and animal studies suggested that the administration of antipsychotics could affect brain morphology (Wang et al., 2004; Lieberman et al., 2005). It is possible that long-term medication with antipsychotics also affects microstructure of the WM in schizophrenics. The longitudinal animal studies may clarify this issue.

In conclusion, we confirmed decreased FA in schizophrenics, compared to controls in the widespread WM areas in a Japanese sample. We found that age-dependent FA decline was more pronounced in chronic schizophrenics compared to controls, and that such FA decline was significantly correlated with duration of illness in patients. These observations suggest that decreased FA values in schizophrenia might be attributable, at least in part, to progressive changes in the WM after the onset of the illness.

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Gene expression in the peripheral leukocytes and association analysis of PDLIM5 gene in schizophrenia

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Abstract

PDLIM5 modulates neuronal calcium signaling, co-localizes with synaptic vesicles of neurotransmitters and positive association between its gene and schizophrenia was reported but its relation is still ambiguous. The differential expression of the PDLIM5 gene both in the brain and in the lymphoblasts has been found in schizophrenia compared to control subjects. In this study, we measured the expression level of the PDLIM5 gene transcripts in the peripheral leukocytes from 19 medication-free and 21 chronically medicated schizophrenic patients as well as age- and sex-matched control subjects using a quantitative real-time PCR method. The mRNA levels of the PDLIM5 gene in the leukocytes of medication-free schizophrenic patients were significantly higher than those of control subjects. On the other hand, our group has previously shown that its mRNA expression in the leukocytes of medication-free major depressive patients was significantly lower compared with controls. There was no difference in the PDLIM5 mRNA levels between chronic schizophrenic patients with antipsychotic medication and their controls. Further, we failed to find any genetic association between the PDLIM5 gene and schizophrenia with six single nucleotide polymorphics (SNPs) of the PDLIM5 gene in Japanese subjects (279 subjects each) and there was no significant relation between PDLIM5 gene and schizophrenia with the haplotype analysis ($P = 0.48$), either. We suggest that the higher expression levels of the PDLIM5 mRNA in the peripheral leukocytes may be a candidate marker for medication-free schizophrenic patients.

Keywords: PDLIM5; Gene expression; Leukocytes; Association analysis; Schizophrenia

PDLIM5 is an intermediate protein that has been shown to regulate intracellular calcium levels by linking calcium channel and protein kinase C (PKC) [2,3,16]. PDLIM5 is ubiquitously expressed and its cellular localization in the brain is identical to Synapsin which is known to be involved in the neurotransmitter release [16]. The PDLIM5 gene lies on chromosome 4q22, a locus previously reported to be linked with schizophrenia [13,19]. While Kato et al. failed to find any association between the PDLIM5 gene and schizophrenia [15], Horiuchi et al. found a significant association between them [6]. It was reported that the expression level of PDLIM5 mRNA was significantly increased in the postmortem brain tissues of patients with schizophrenia, bipolar disorder and major depression, but was decreased in the immortalized lymphoblastoid cell lines derived from patients with schizophrenia and bipolar disorder [10,11]. Our group has recently shown that levels of mRNA expression in the peripheral leukocytes of the PDLIM5 gene were significantly lower in medication-free major depressive patients compared with controls [8].

The expressional alterations of genes in the peripheral blood lymphocytes and leukocytes have been reported to indicate the changes of the central nervous systems in schizophrenia and

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Table 1a
Demographic data for medication-free schizophrenic patients studied in PDLIM5 mRNA expression analysis ($N = 19$)

	Age (y.o)	Gender	Age at onset (years)	BPRS score	Family history of schizophrenia in first-degree relative
S1	25	M	22	64	+
S2	24	M	24	42	–
S3	24	M	24	31	–
S4	27	M	24	37	–
S5	36	M	36	34	–
S6	39	M	38	59	–
S7	27	M	26	58	–
S8	20	F	19	46	–
S9	23	F	23	48	–
S10	34	F	31	36	–
S11	47	F	47	30	–
S12	15	F	13	30	+
S13	26	F	21	100	–
S14	23	M	23	31	–
S15	28	M	25	63	–
S16	47	F	47	37	–
S17	37	F	21	36	–
S18	30	F	25	41	–
S19	45	F	43	36	+

The age (years old: y.o) represents the age of the subject when the leukocytes were drawn. M: male, F: female, '+' indicates that at least one of the first-degree relatives has schizophrenia.

major depressive disorder [7,8,9,17,21]. In this study, we measured the PDLIM5 mRNA expression levels in the peripheral leukocytes in unmedicated and medicated schizophrenic patients as well as in control subjects, using a quantitative real-time PCR method. In addition, we examined the genetic case-control study of the PDLIM5 gene with schizophrenia in Japanese subjects comprising of 279 patients with schizophrenia and 279 controls.

All patients and controls were biologically unrelated Japanese. The diagnosis of schizophrenia was made by at least two experienced psychiatrists according to DSM-IV criteria [1]. Clinical symptoms were evaluated by the Brief Psychiatric Rating Scale scores (BPRS) [20] when blood samples were taken. Age- and sex-matched controls were in good physical health without a history of any psychiatric or serious somatic diseases and taking any medication during the sample collection period. Probands who had first-degree relatives with psychiatric disorders were excluded from the control subjects.

For the measurement of expression levels of the PDLIM5 mRNA, the subjects consisted of 19 medication-free patients with schizophrenia (subject number S1–S19, Tables 1a and 1b) (14 first-episode and drug-naïve schizophrenic patients, 5 schizophrenic patients without antipsychotic treatment for at least 2 months; 9 males and 10 females, mean age: 30.4 ± 9.3), 19 age- and sex-matched controls (9 males and 10 females, mean age: 30.6 ± 8.6), 21 chronically treated patients with schizophrenia who were stably controlled under the same amount dosage of antipsychotics for at least 3 months (subject number S20–S40, Tables 2a and 2b) (13 males and 8 females, mean age: 47.7 ± 11.3) and 21 age- and sex-matched controls (mean age: 47.7 ± 11.1).

For the genetic association study, we used DNA samples from 279 in patients (189 male and 90 female; mean age: 51.3 ± 13.7 years) with schizophrenia from 13 psychiatric hospitals in the neighboring area of Tokushima Prefecture in Japan (population: about 820,000). Age- and sex-matched controls were selected from volunteers after assessing the psychiatric problems (189 male and 90 female; mean age: 51.4 ± 12.0) for the association and haplotype-based case-control study.

All subjects signed written informed consent to participate in the expression and genetic association studies approved by the institutional ethics committees.

Table 1b
PDLIM5 mRNA expression in medication-free schizophrenic ($N = 19$) and control subjects ($N = 19$)

	Male ($N = 9$)	Female ($N = 10$)	Total ($N = 19$)
Schizophrenia (S1–S19)			
Age	28.1 ± 5.6	32.4 ± 11.5	30.4 ± 9.3
The PDLIM5 mRNA expression before treatment	1.13 ± 0.3	1.29 ± 0.3	$1.21 \pm 0.3^*$
Control			
Age	27.6 ± 4.8	33.4 ± 10.4	30.6 ± 8.6
The PD1IM5 mRNA expression	0.95 ± 0.2	1.03 ± 0.4	1.00 ± 0.3

The mean PDLIM5 mRNA levels in the peripheral leukocytes from medication-free schizophrenia patients were significantly higher than those of age- and sex-matched controls (Mann–Whitney U test: $P = 0.023$); $^*P < 0.05$. No correlation between PDLIM5 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.38$).

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Table 2a
Demographic data for chronic schizophrenic patients studied in PDLIM5 mRNA expression analysis ($N = 21$)

	Age (y.o)	Gender	Medication	BPRS Score
S20	57	M	QTP 75 mg, LP 150 mg, CP 300 mg	55
S21	56	M	Ris 6 mg	29
S22	56	M	Ris 5 mg, QTP 200 mg, sulpiride 150 mg	44
S23	60	M	Ris 8 mg, LP 20 mg	67
S24	57	M	HPD 9 mg, BPD 9 mg propericyazine 60 mg	52
S25	40	M	Ris 12 mg	33
S26	46	M	Ris 6 mg, HPD 9 mg, sultopride 900 mg	49
S27	45	M	BPD 9 mg, clocaprarmine 75 mg	59
S28	31	M	BPD 2 mg, HPD 1 mg, LP 15 mg Perospirone 24 mg	49
S29	49	F	Ris 6 mg, HPD 6 mg, CP 20 mg, HPD decanoate 150 mg	33
S30	53	F	HPD 2.25 mg, sulpride 150 mg	33
S31	65	F	HPD 4.5 mg, CP 37.5 mg	47
S32	51	F	Olz 10 mg	23
S33	43	F	Ris 6 mg, zotepine 50 mg	45
S34	54	F	Olz 20 mg, LP 50 mg	38
S35	54	M	Ris 12 mg, zotepine 150 mg timiperone 6 mg	42
Sc36	25	M	Ris 9 mg, perospirone 16 mg	39
Sc37	49	M	Ris 12 mg, LP 150 mg	54
Sc38	23	M	Ris 12 mg, LP 150 mg	38
Sc39	35	F	Olz 20 mg	33
Sc40	53	F	QTP 400 mg	27

The age (years old: y.o) represent the age of the subject when the leukocytes were drawn. M: male, F: female, Olz: olanzapine, Ris: risperidone, HPD: hapoperidol, BPD: bromperidol, LP; levom epromazine.

Total RNA was extracted from the peripheral leukocytes using the PAX gene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations. One microgram of total RNA was used for cDNA synthesis by QuantiTect Reverse Transcription Kit (Qiagen) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, DE, USA). Expression of the PDLIM5 gene transcript was quantified by real-time PCR with the TaqMan Gene Expression Assay (Applied Biosystems, CA, USA). Primers and probes (Hs00179051_m1) were purchased from Applied Biosystems as well as Horiuchi's group [6]. GAPDH gene expression was used as an internal control and measurement of threshold cycle (Ct) was performed in triplicate. Data were collected and analyzed with Sequence Detector Software version 2.1 (Applied Biosystems) and the standard curve method. Relative gene expression was calculated as the ratio of PDLIM5 to GAPDH gene and the mean of the three replicate measures was assigned to each individual. Almost all of blood samples were taken in the morning before lunch. The expression of the PDLIM5 mRNA was not changed among blood samples collected at several points during the day time or over several weeks in the same control subjects.

Genotyping was performed using commercially available TaqMan probes (C_2095059_10, C_16015055_20, C_3226622_10, C_16015313_10, C_1569781_10, C_11567561_10) with Applied Biosystems 7500 Fast Real Time PCR System according to the protocol recommended by the manufacturer (Applied Biosystems). We selected six single nucleotide polymorphic (SNP) markers for genotyping according to linkage disequilibrium (LD) and haplotype blocks in the PDLIM5 gene region [6]. Two SNPs (rs10008257, rs2433320) in the 5′-flanking region and four SNPs left in the genomic region are covered about 169-kb in the whole 214-kb of the PDLIM5 gene. The heterozygocities of four of these six SNPs, rs10008257, rs2433320, rs2433327 and rs2452600 in Japanese population are reported as 0.39, 0.18, 0.26 and

Table 2b
PDLIM5 mRNA expression in chronic treated schizophrenic ($N = 21$) and control subjects ($N = 21$)

	Male ($N = 13$)	Female ($N = 8$)	Total ($N = 21$)
Schizophirenia (S20–S40)			
Age	46.1 ± 12.7	50.4 ± 8.7	47.7 ± 11.3
The PDLIM5 mRNA expression	0.78 ± 0.2	0.93 ± 0.2	0.83 ± 0.2
Control			
Age	46.2 ± 12.3	50.1 ± 9.0	47.7 ± 11.1
The PD1IM5 mRNA expression	0.90 ± 0.3	1.14 ± 0.4	1.00 ± 0.3

The mean PDLIM5 mRNA levels in the peripheral leukocytes from schizophrenia patients who has been treated with antipsychotic drugs for many years were not different from controls' (Mann–Whitney U test: $P = 0.16$). No correlation between PDLIM5 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.82$).

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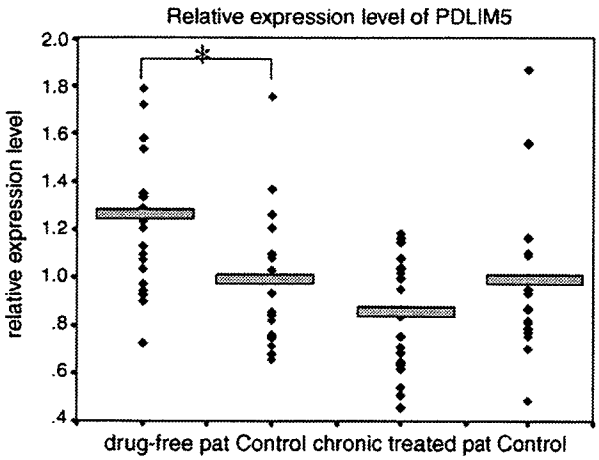

Fig. 1. Relative expression levels of PDLIM5 in the peripheral leukocytes in schizophrenic patients and control subjects. Compared with the normal control group, the mean PDLIM5 mRNA level in the leukocytes of medication-free schizophrenic patients ($N = 19$) was significantly higher (patients: 1.21 ± 0.29, controls: 1.00 ± 0.29, Mann–Whitney U test: $P = 0.023$). The mean PDLIM5 mRNA level in the leukocytes of chronic schizophrenic patients ($N = 21$) showed no significant difference compared with controls (patients: 0.83 ± 0.23, controls: 1.00 ± 0.32, Mann–Whitney U test: $P = 0.16$).

0.34, respectively. The heterozygocities of the other two SNPs, rs12641023 and rs14082, are not reported.

Statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Expressional differences between patients and control subjects were calculated using the Mann–Whitney U test. Spearman correlation coefficients were used to evaluate the correlations between PDLIM5 mRNA levels and BPRS scores. Two-way ANOVA was performed to determine the independent and combined effects of age and the expression of PDLIM5 between groups. Allele and genotype frequencies of patients and control subjects were compared using Fisher's exact test. The SNPAlyze 3.2 Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, LD, and permutation P-values. Pair-wise linkage disequilibrium indices, D' and r2, were calculated in the control subjects. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± standard deviation.

Relative expression levels of PDLIM5 mRNA in 19 medication-free patients (S1–S19) were 1.21 ± 0.29 in the range of 0.73–1.79, while 1.00 ± 0.29 (range: 0.66–1.75) in healthy volunteers, showing a statistical difference (Mann–Whitney U test: $P = 0.023$, Fig. 1). Mean BPRS scores was 45.2 ± 17.4. No correlation between PDLIM5 mRNA levels and baseline BPRS scores were observed (Spearman's correlation efficient: $P = 0.38$). There was no significant expressional difference of PDLIM5 mRNA levels either between males and females or between genotypes of the single nucleotide polymorphism (rs2433320) both in patients with schizophrenia and in control subjects.

Relative PDLIM5 mRNA level was 0.83 ± 0.23 (0.46–1.18) in 21 chronically treated patients (S20–S40), while 1.00 ± 0.32 (0.49–1.87) in healthy volunteers, showing no significant statistical difference (Mann–Whitney U test: $P = 0.16$; Fig. 1). Mean chlorpromazine-equivalent doses were 932.1 ± 510.5 mg/day and mean duration of treatment was 23.5 ± 10.7 years and mean BPRS scores was 43.1 ± 10.8. No significant relationship between PDLIM5 mRNA levels and BPRS scores was observed (Spearman correlation efficient: $P = 0.71$). There was no significant expressional difference of PDLIM5 mRNA levels either between males and females or between genotypes of the single nucleotide polymorphism (rs2433320) both in patients with schizophrenia and in control subjects.

There were no significant deviations in all six SNPs from Hardy-Weinberg equilibrium in either patients or control subjects. Allele and genotype frequencies of the six SNPs are shown in Table 3. There were no associations between these SNPs and schizophrenia neither in the allelic frequency nor in the genotypic distributions. Although both rs2433320-rs2443327 and rs12641023-rs14082 were in a tight LD ($D' = 0.936$, 0.968, each), permutation test showed no significant difference in estimated frequencies of these haplotypes between the controls and patients (global permutation $P = 0.58$, 0.45; each, Table 4). Haplotypes of six SNPs were evaluated, but no significant difference was observed in frequencies of any estimated haplotype or in distributions of all estimated haplotypes between the controls and patients (global permutation $P = 0.48$).

The present study is the first report on the PDLIM5 gene expression in the peripheral leukocytes in schizophrenia. The mean PDLIM5 mRNA levels in the peripheral leukocytes from medication-free schizophrenia patients were significantly higher than those of age- and sex-matched controls. Altered mRNA expression in the peripheral lymphocytes could reflect the altered metabolism of brain cells [4]. Our result is consistent with the result of higher expression in the postmortem brains from schizophrenic patient but not with the result of lower expression in the lymphoblastoid cells derived from schizophrenic patients [10,11]. The differences of the mRNA expression between studies may be partly attributed to the difference in the materials. When using lymphoblastoid cells, the effect of virus infection or chromosomal alterations during culture must be taken into account [12]. On the other hand, the mRNA expression level of PDLIM5 gene was not significantly higher in chronically treated schizophrenics compared with that of controls. This finding in the chronic patients may be a consequence of pharmacological effects of antipsychotics or clinical improvement. This result suggests that expression of PDLIM5 mRNA may not be trait-oriented but state-related change. To confirm whether the expression of this gene is a state marker, a follow-up investigation is needed in the same patients before and after treatment.

The pathophysiological mechanism remains unknown, but we speculate that the higher expression of PDLIM5 is related with putatively elevated Ca^{2+} signaling in schizophrenia. It has been suggested that abnormalities in Ca^{2+} signaling was associated with molecular etiology of schizophrenia. Regulator of G protein signaling-4 (RGS4) and B-cell limphoma/leukaemia-2 gene (Bcl-2) which reduce free Ca^{2+} in a cell have been found to be down regulated in the temporal cortex of schizophrenic patients [14,18]. It was reported that there was high levels of free intracellular Ca^{2+} in platelets of schizophrenic patients

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Table 3
Genetic studies of PDLIM5 gene with schizophrenia in case-control samples

Group	Genotype			n	Hardy-Weinberg equilibrium	P-value	Allele		P-value
ra1 0008257	A/A	A/G	G/G				A	G	
sch	42	127	105	274	0.823	0.471	211	337	0.804
cont	34	140	102	276	0.229		208	344	
rs2433320									
sch	7	75	197	279	0.858	0.601	89	469	0.871
cont	11	70	198	279	0.205		92	466	
rs2433327	T/T	T/C	C/C				T	C	0.833
sch	169	88	16	273	0.414	0.917	426	120	
cont	164	92	15	271	0.788		420	122	
rs2452600	T/T	T/C	C/C				T	C	
sch	54	125	96	275	0.306	0.232	233	317	0.080
cont	68	130	81	279	0.325		266	292	
rsl2641023	A/A	A/G	G/G				A	G	
sch	51	126	93	270	0.555	0.497	228	312	0.295
cont	42	131	103	276	0.924		215	337	
rs14082	A/A	A/G	G/G				A	G	
sch	58	124	91	273	0.243	0.302	240	306	0.141
cont	45	125	103	273	0.582		215	331	

sch: Schizophrenia, cont: control subjects. P-values are calculated by Fisher s exact test.

[22]. PDLIM5 regulates intracellular calcium levels by linking calcium channel and protein kinase C [2,3,16]. The levels of PDLIM5 might be up-regulated both in the brain and in the peripheral leukocytes in patients with schizophrenia in response to increased intracellular calcium levels. It has been demonstrated that antipsychotic drugs block IP3-induced release of Ca^{2+} [23] and Ca^{2+} dependence of PKC is well known [5]. So antipsychotic medication might normalize the up-regulation of PDLIM5 expression in schizophrenia by reducing Ca^{2+} signaling.

PDLIM5 may be involved in other mental disorders. Iwamoto et al. reported that expression level of PDLIM5 was significantly and commonly increased in the postmortem brain tissues of patients with schizophrenia, major depression and bipolar disorder [11]. However, we have already shown that mean PDLIM5 mRNA level in the peripheral leukocytes of medication-free patients with major depression was significantly lower than in control subjects [8]. Therefore, the higher expression of this gene in the peripheral leukocytes of medication-free patients with schizophrenia may be disease-specific and not due to non-specific stress of psychiatric condition. Further investigations of other psychiatric diseases including bipolar disorder are needed.

Table 4
Linkage disequilibrium (LD) indices (lower left are r2, upper right are D')

	rs10008257	rs2433320	rs2443327	rs2452600	rs12641023	rs14082
rs10008257		0.37227	0.44147	0.28294	0.12734	0.15919
rs2433320	0.01632		0.9364	0.50709	0.37209	0.40839
rs2443327	0.03427	0.57719		0.54423	0.43945	0.45693
rs2452600	0.0447	0.05573	0.09541		0.19114	0.18089
rs12641023	0.00626	0.04284	0.08854	0.02152		0.96845
rs14082	0.01002	0.05068	0.09508	0.01918	0.93062	

Horiuchi et al. reported that there were significant association between polymorphisms (rs2433320 and rs2433322) of PDLIM5 gene and schizophrenia. Their group also showed that the different alleles of the rs2433320 showed different DNA–protein complexes on electrophoreic mobility shift assay and GA heterozygotic genotype might have higher transcriptional activity in schizophrenia [6]. However, our result showed that there was not significant association between schizophrenia and six polymorphisms of PDLIM5 gene, including rs2433320, and this result is consistent with a previous study with a large number of subjects ($n = 562$) [15]. In addition, neither patients nor controls showed a significant difference of the PDLIM5 mRNA expression in the peripheral leukocytes between GG and GA genotypes of this SNP in our subjects although the typeII error was not denied.

In conclusion, our investigation revealed that the mean PDLIM5 mRNA levels in medication-free schizophrenic patients were significantly higher compared to those in controls and the chronic schizophrenic patients with antipsychotic treatment for many years showed almost the same expression levels as healthy control levels. There were no associations between schizophrenia and PDLIM5 gene. These results suggest that the higher expression levels of PDLIM5 mRNA in the leukocytes may be a candidate marker for medication-free schizophrenic patients. Further studies are necessary to confirm the present results.

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A possible association between the Val158Met polymorphism of the catechol-*O*-methyl transferase gene and the personality trait of harm avoidance in Japanese healthy subjects

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Abstract

Catechol-*O*-methyltransferase (COMT) is an enzyme that degrades various biogenic amines, which have been hypothesized to be associated with personality traits. We investigated a possible relationship between the COMT Val158Met polymorphism and personality traits assessed by the Temperament and Character Inventory (TCI) in 139 healthy subjects in a Japanese population. The number of Met alleles of the COMT Val/Met genotype tended to relate to harm avoidance (HA) scores parametrically, while no significant difference was observed between genotype groups in either novelty seeking, reward dependence, persistence, self-directedness, cooperativeness or self-transcendence. These results suggest that the Val/Met polymorphism of the COMT gene may play a role in HA in Japanese population.

Keywords: Catechol-*O*-methyltransferase (COMT); Temperament and Character Inventory (TCI); Harm avoidance; Polymorphism

Genetic factors significantly contribute to the determination of human personality traits, although environmental influence is also important. Personality traits assessed by self-report questionnaires show moderate heritability [6]. Such inheritance is ultimately attributable to functional variants of the genes programming brain development and function [5]. Catechol-*O*-methyl transferase (COMT) is an enzyme involved in monoamine metabolism, and a common single nucleotide polymorphism (SNP) in the COMT gene, producing an amino acid substitution of methionine (Met) to valine (Val) at position 108/158 (Val158Met), affects dopamine regulation in the prefrontal cortex [19]. This polymorphism impacts on the stability of the enzyme, such that the Val allele is associated with significantly higher enzyme activity than the Met allele [4]. Several studies have revealed that the Val allele is associated with poorer performances, compared with the Met allele, in cognitive tasks of frontal function such as the Wisconsin Card Sorting Test (WCST) and N-back task [7,8]. The underlying mechanism of such behavioral differences may be related to lower prefrontal dopamine levels arising from the higher dopamine catabolism mediated by the Val allele [4,23]. Thus, it is likely that the Val/Met polymorphism of the COMT gene could be associated with a personality trait; however, the relationship between the Val/Met polymorphism of the COMT gene and the personality traits using the Temperament and Character Inventory (TCI) has not been studied in Japanese population [1,2,9,14–16,21,22]. In this study, we examined the relationship between the Val158Met of the COMT gene and the personality traits measured by TCI in Japanese healthy subjects.

One hundred and thirty-nine healthy subjects participated in the study. A Japanese version of TCI, a full version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [20,24]

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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

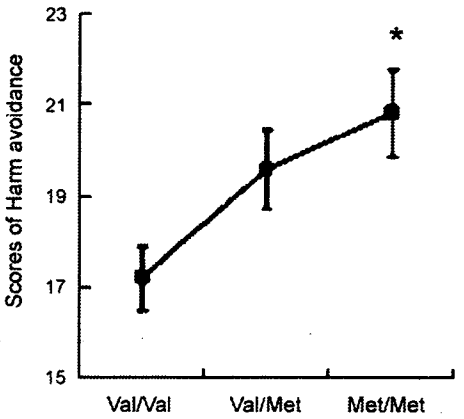

Fig. 1. Harm avoidance and COMT Val/Met genotype. Scores of harm avoidance in healthy individuals with Val/Val ($n=77$), Val/Met ($n=45$) and Met/Met ($n=17$) genotypes are shown (mean ± S.D.). *$P<0.05$, significant difference compared with Val/Val.

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 ± 23.0% in patients and 65.9 ± 19.7% in controls; $t = 0.5$, d.f. = 27, $p = 0.61$) or right (64.2 ± 27.4% in patients and 65.4 ± 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.

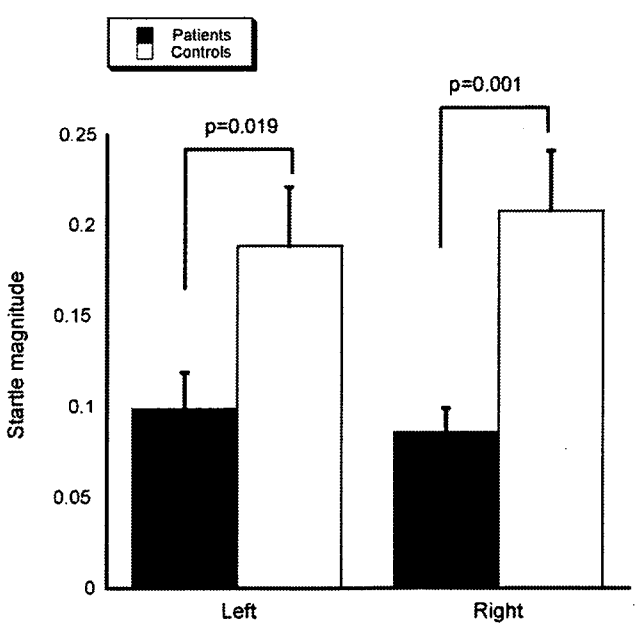

Fig. 1. Startle magnitude in patients and controls.

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

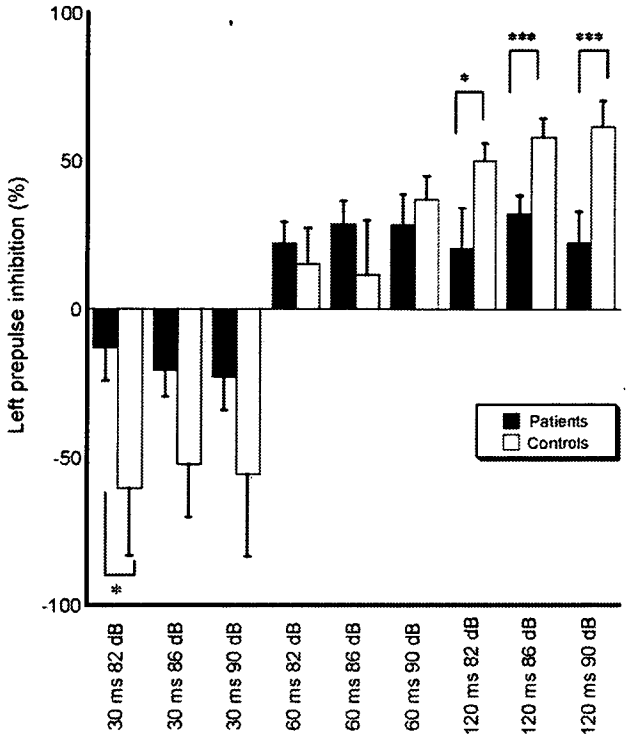

Fig. 2. Prepulse inhibition (PPI) in patients and controls. Records from the left side are shown. Error bars represent standard errors. $*p < 0.10$, $***p < 0.01$.

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.

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