brain: (1) the choice of an appropriate brain region for investigation; (2) the heterogeneity of cell types within brain tissue; (3) the reliance on relatively small samples; and (4) the impact of cause of death and/or postdeath handling of the tissues on gene expression [Marcotte et al., 2003]. Thus, the use of postmortem brain tissue is compounded by a range of confounding factors (age, race, gender, different microarray platforms, and analysis methods) and may be the cause of the relative lack of gene/transcript-level consistency among expression studies. To overcome some of these problems, several groups have considered the use of lymphoblasts rather than the postmortem brain [Matigian et al., 2008; Slonimsky et al., 2010; Yamamori et al., 2011; Yasuda et al., 2011]. Lymphoblasts are useful for schizophrenia researchers because blood-based tissue (lymphoblasts) can be obtained with ease from living subjects, which allows larger case–control studies with optimal matching of key variables (age, sex, and race). In addition, immortalized lymphoblasts in culture are considered an effective tool for studying cells in the absence of the effect of antipsychotic treatments and duration of illness, both of which could mask the genetic differences in RNA expression. Thus, lymphoblasts could be good tool to investigate the impact of a gene in the absence of the impact of any confounding factors. On the other hand, there were some demerits of using lymphoblasts. In immortalized lymphocytes, it might be difficult to observe the effects of genes on their neuron-specific functions, for example, the effects of genes on glutamate and dopamine release and on the formation of synaptic vesicles. When isolation and immortalization procedures of lymphocytes from blood were performed or immortalized lymphocytes were grown in culture media, a genetic mutation might be inserted into genomic DNA in the cultured lymphoblasts and alter DNA sequences. It remains still controversial whether immortalized lymphocytes are an appropriate alternative to neuronal tissue, because there was a little evidence of analysis using immortalized lymphocytes from patients with schizophrenia. In this study, the difference in the association of gene expression with genetic variants between previous study and present study could be explained by the difference in the gene expression profile between immortalized lymphoblast and postmortem brain tissue. Other possible factors contributing to differences in association between studies could be a difference in the SNPs and haplotypes investigated or ethnic differences between Japanese and Caucasian populations.

Smith et al. [2011] performed mutation searches of all four exons of *NRGN* gene in 14 Caucasian subjects with schizophrenia and of the cording exons of *NRGN* gene in 1,113 Bulgarians individuals, 699 of whom had schizophrenia. However, they did not find any novel common polymorphism in the region. Thus, we did not perform a systematic mutation search in this study because there has been no novel common genetic variant in the region. If we perform sequencing and find a novel rare polymorphism, we cannot analyze association between the rare polymorphism and gene expression for only a small number of individuals with rare variant. A genetic variant, particularly a SNP not listed in the HapMap database, that is likely to be more strongly associated with schizophrenia may exist in the rs12807809–rs12278912 haplotype region. Sequencing the entire gene in individuals with risk haplotype in comparison with the protective haplotype carriers with larger sample sizes could provide further information underlying the genomic mechanism for this risk haplotype.

There are several limitations to interpreting our results. Because a number of statistical analyses supported the association of the *NRGN* gene and schizophrenia, such as genotypic and allelic associations for five SNPs (total 5×2), haplotype analysis using a window fashion analysis (total 10) and expression analysis for three individual haplotypes (total 3×4), a correction for multiple testing should be considered. In this study, the overall number of genetic association tests was 32; however, not all tests were independent, and several hypotheses were included. Thus, Bonferroni correction, a method to correct for multiple independent tests for one hypothesis, might not be appropriate. The consensus how to correct such multiple testing has not been reached in this research field. Thus, we applied SNPSpD correction for genotypic and allelic association analysis, permutation method for haplotype analysis and Bonferroni correction for expression analysis (three tests). However, even though we applied these methods of correcting such multiple testing, they might cause false positive results. We did not control for geographical variation of control origin because there is little possibility for ethnic/genetic difference among four geographical regions for feature of homogeneous race in Japan [Yamaguchi-Kabata et al., 2008]. Our significant results may be derived from sample bias owing to population stratification and non-sex-matched samples. In the present study, our results support an association between the *NRGN* gene and schizophrenia. We suggest that the functional haplotype of the *NRGN* gene, which is associated with *NRGN* expression, could be related to the pathogenesis of schizophrenia.

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The *p250GAP* Gene Is Associated with Risk for Schizophrenia and Schizotypal Personality Traits

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Abstract

Background: Hypofunction of the glutamate *N*-Methyl-d-aspartate (NMDA) receptor has been implicated in the pathophysiology of schizophrenia. p250GAP is a brain-enriched NMDA receptor-interacting RhoGAP. p250GAP is involved in spine morphology, and spine morphology has been shown to be altered in the post-mortem brains of patients with schizophrenia. Schizotypal personality disorder has a strong familial relationship with schizophrenia. Several susceptibility genes for schizophrenia have been related to schizotypal traits.

Methods: We first investigated the association of eight linkage disequilibrium-tagging single-nucleotide polymorphisms (SNPs) that cover the *p250GAP* gene with schizophrenia in a Japanese sample of 431 schizophrenia patients and 572 controls. We then investigated the impact of the risk genetic variant in the *p250GAP* gene on schizotypal personality traits in 180 healthy subjects using the Schizotypal Personality Questionnaire.

Results: We found a significant difference in genotype frequency between the patients and the controls in rs2298599 ($\chi^2 = 17.6$, $p = 0.00015$). The minor A/A genotype frequency of rs2298599 was higher in the patients (18%) than in the controls (9%) ($\chi^2 = 15.5$, $p = 0.000083$). Moreover, we found that subjects with the rs2298599 risk A/A genotype, compared with G allele carriers, had higher scores of schizotypal traits ($F_{1,178} = 4.08$, $p = 0.045$), particularly the interpersonal factor ($F_{1,178} = 5.85$, $p = 0.017$).

Discussion: These results suggest that a genetic variation in the *p250GAP* gene might increase susceptibility not only for schizophrenia but also for schizotypal personality traits. We concluded that the *p250GAP* gene might be a new candidate gene for susceptibility to schizophrenia.

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Introduction

Schizophrenia is a common and complex psychiatric disease. The lifetime morbidity rate is 0.5–1.0% across distinct populations. Family, twin and adoption studies of schizophrenia have indicated that there are strong genetic factors and have estimated the rate of schizophrenia heritability at 80% [1,2]. Although genes implicated in the pathogenesis of schizophrenia have been found using several approaches, such as through association studies of candidate genes, genome-wide association studies (GWAS), copy number variation (CNV) studies and pedigree studies [3,4], the exact genetic factors of this complex disease remain to be explained.

Hypofunction of the glutamate *N*-Methyl-d-aspartate (NMDA) receptor is strongly implicated in the pathophysiology of schizophrenia. NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, mimic symptoms of the disorder in humans and exacerbate symptoms in patients with schizophrenia [5]. These NMDA receptor antagonists induce schizophrenia-like symptoms in humans. Preclinically, they have been shown to

induce similar symptoms and to induce neural circuitry changes reminiscent of schizophrenia [6]. The ability of these NMDA receptor antagonists to induce a schizophrenia-like phenotype supports the concept that schizophrenia may be the result of reduced or abnormal functioning of NMDA receptors. Altered NMDA receptor binding density in several brain regions, such as in the anterior cingulate cortex, has been reported in schizophrenia [7,8]. The NR2 subunits of the NMDA receptor are spatially and developmentally regulated, and they provide an important level of receptor regulation [9,10]. NR2A and NR2B are the predominant subunits in the cortex, striatum and hippocampus [11,12,13]. In particular, these three areas are closely associated with the pathology of schizophrenia and with the neural circuits within and between these regions [14]. In patients with schizophrenia, alterations have been observed in the NR2 subunit mRNA and protein in the prefrontal cortex, including a reduction in NR2A mRNA and NR2B protein levels [15,16]. Additionally, the NR2B subunit mRNA levels were increased in the hippocampus [17]. Therefore, different expression of NR2 subunits could play an important role in the pathophysiology of schizophrenia.

The NMDA receptor regulates activity-dependent spine morphological plasticity by modulating the actin cytoskeleton [18]. As the key regulators of actin cytoskeleton dynamics, the Rho family of GTPases, including RhoA, Cdc42, and Rac1 and their regulators, play an important role in NMDA receptor-mediated spine morphogenesis [18,19]. In our previous study, we identified the *p250GAP* gene (also known as *p200RHOGAP*, *GRIT*, *KIAA0712*, *RICS*, or *ARHGAP32*: OMIM 608541) as a novel NMDA receptor-interacting RhoGAP [20,21,22,23]. This gene spans approximately 56.17 kb of the genomic DNA and is located on chromosome 11q24.3. p250GAP is highly enriched in the central nervous system, is concentrated in the post-synaptic densities in neurons and is co-localized with the NR2B subunit of the NMDA receptor [20]. Knockdown of p250GAP increased spine width and elevated the endogenous RhoA activity in primary hippocampal neurons, suggesting that p250GAP regulates spine morphogenesis through its RhoGAP activity for RhoA [24]. Importantly, p250GAP activity and localization within neurons are regulated by NMDA receptor activity [20,24], suggesting that p250GAP, together with the NMDA receptor, regulates NMDA receptor-mediated spine morphogenesis. Given that neuropathological studies of schizophrenia have shown alterations in spine morphology [25,26], we hypothesized that the *p250GAP* gene may be related to the pathophysiology of schizophrenia. In this study, we investigated the association between the *p250GAP* gene and schizophrenia in a Japanese population using a gene-based approach.

Schizotypal personality disorder (SPD) is one of the schizophrenia spectrum disorders and is characterized by social avoidance, ideas of reference, vagueness, magical thinking, odd speech, illusions and paranoid ideation. The lifetime prevalence of SPD has been estimated at 3.9% [27], making it one of the more common psychiatric disorders. The prevalence rate of SPD in relatives of individuals with schizophrenia (6.9%) was higher than the prevalence rates found either in relatives of individuals with other psychiatric disorders or in mentally healthy subjects [28]. Twin studies have estimated that the heritability of the latent liability to SPD is 61–72% [29,30]. Premorbid SPD is related to the development of schizophrenia [31]. These findings suggest that SPD shares common genetic influences with schizophrenia. The traits of SPD were incorporated in the SPD criteria in the *Diagnostic and Statistical Manual of Mental Disorders*, third edition (DSM-III), and the traits are listed in the DSM-IV-TR on Axis II. These traits can be identified using a well-validated questionnaire, such as the Schizotypal Personality Questionnaire (SPQ) [32]. The heritability rates of three schizotypal trait factors, cognitive/perceptual, interpersonal and disorganization, have been estimated at 40 to 60% [33,34]. We recently demonstrated that a genome-wide genetic variant for schizophrenia in the *ZNF804A* gene was associated with schizotypal personality traits [35]. Additionally, we investigated whether a genetic variant in the *p250GAP* gene was associated with schizotypal personality traits in healthy subjects.

Materials and Methods

Ethics statement

Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was performed in accordance with the World Medical Association's Declaration of Helsinki and approved by the Osaka University Research Ethics Committee.

Subjects

The subjects of our genetic association study were 431 patients with schizophrenia (48.7% male (210 males, 221 females), mean age ± SD was 49.7±15.4 years) and 572 healthy controls (46.7% male (267 males, 305 females), mean age ± SD was 61.9±20.4 years). The sex ratio did not differ significantly between the groups ($\chi^2 = 0.41$, $p = 0.52$), but the mean age was significantly different ($z = -11.49$, $p < 0.001$). The subjects were all biologically unrelated and were Japanese. The subjects were recruited from both outpatient and inpatient units at Osaka University Hospital and other psychiatric hospitals. Each patient with schizophrenia had been diagnosed by at least two trained psychiatrists by unstructured clinical interviews, according to the criteria of the DSM-IV. When the diagnosis of the two trained psychiatrists was discordant, they discussed the diagnosis. When the diagnostic disputes were resolved and the patient was diagnosed as schizophrenic, we included the patient. When the diagnostic disputes were not resolved by discussion or the patient was not diagnosed as schizophrenia, we excluded the patient. Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals with current or past contact with psychiatric services, with experience with psychiatric medications or who were not Japanese. We did not assess the controls for their family history of mental disorders, such as schizophrenia, bipolar disorder, or major depressive disorder. The ethnicity was determined by self-report and was not confirmed by genetic analyses.

Data for the schizotypal personality trait analysis were available for 180 healthy subjects [48.3% male (87/93), mean age ± SD: 36.5±11.5 years]. The subjects were included in the genetic association analysis. The subjects included in the analysis met additional criteria. Psychiatrically, medically and neurologically healthy controls were evaluated using the Structured Clinical Interview for DSM-IV-Non-Patient Edition (SCID-I/NP) to exclude individuals who had received psychiatric medications or who had first- or second-degree relatives with psychiatric disorders. Additionally, subjects were excluded from this study if they had neurological or medical conditions that could have potentially affected their central nervous system, such as atypical headaches, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation.

SNP selection, genotyping and genomic sequencing

This study was designed to examine the association between the *p250GAP* gene and schizophrenia by tagging single-nucleotide

polymorphisms (SNPs) in the *p250GAP* gene and its flanking regions (±5 kb). Of the 31 SNPs in the *p250GAP* gene and flanking regions, we selected eight tagging SNPs using the TAGGER algorithm (Paul de Bakker, http://www.broad.mit.edu/mpg/tagger) with the criteria of r^2 greater than 0.5 in 'pairwise tagging only' mode and a minor allele frequency (MAF) greater than 5%. The selection was implemented in Haploview 4.2 using HapMap data release 24/PhaseII Nov 08, on NCBI B36 assembly, dbSNP b126 (Japanese in Tokyo (JPT), Chr 11: 128,338,052..128,404,222) (Table S1). The eight tagging SNPs were rs493172, rs10893947, rs2276027, rs3796668, rs581258, rs3740829, rs546239 and rs2298599. The markers are shown in Table 1; the orientation and the alleles are reported on the genomic minus strand. The positions of the eight SNPs analyzed in the present study and the LD relationships between the SNPs in a HapMap JPT population are shown in Figure 1. Venous blood was collected from the subjects. Genomic DNA was extracted from the whole blood using standard procedures. The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems; Foster City, California, USA) as previously described [36,37]. Detailed information on the PCR conditions is available upon request. Genotyping call rates were 99.3% (rs493172), 98.9% (rs10893947), 99.1% (rs2276027), 99.7% (rs3796668), 98.4% (rs581258), 99.2% (rs3740829), 98.5% (rs546239) and 99.3% (rs2298599). No deviations from the Hardy-Weinberg equilibrium (HWE) in the examined SNPs were detected ($p>0.05$). Additionally, with 48 subjects with schizophrenia, we confirmed a SNP significantly associated with schizophrenia, genotyped by the TaqMan method, using direct DNA sequencing. These subjects were included in the genetic association analysis. The genomic regions were amplified by PCR using a pair of primers for rs2298599, 5′- AAGTCAGCCCA-GACTCTCCA -3′ and 5′- GAGGGAGGAAGGGATTTTTG -3′. PCR for each sample was carried out in a total volume of 40 µl using a Gene Amp® PCR System 9700 (Applied Biosystems, CA, U.S.A). The PCR cycling conditions were 94°C for 10 minutes, 30 cycles at 94°C for 1 minute, 60°C for one minute and 72°C for 1 minute, followed by an incubation at 72°C for 10 minutes. The PCR products were purified using a QIA quick® PCR Purification Kit (QIAGEN, CA, USA), and the purification products were sequenced using a Big Dye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Cycle sequencing conditions were 96°C for 2 minutes, 25 cycles of 96°C for 20 seconds, 50°C for 30 seconds and 60°C for 2 minutes, using a Gene Amp® PCR System 9700. The PCR products from the cycle sequencing were purified using a Big Dye® XTerminator™ Purification Kit (Applied Biosystems, CA, U.S.A.), and they were sequenced using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, CA, U.S.A.). The sequencing was checked with SEQUENCHER ver. 4.7 (Gene Codes, U.S.A.).

Schizotypal personality trait analysis

To assess schizotypal personality traits, a full Japanese version of the SPQ was administered to healthy subjects [38,39]. The SPQ is a 74-item self-report questionnaire with a "yes/no" response format [40]. All items answered "yes" were scored 1. The SPQ measures nine subscales of specific schizotypal features, which are ideas of reference, odd beliefs/magical thinking, unusual perceptual experiences, suspiciousness/paranoid ideation, social anxiety, no close friends, constricted affect, eccentric/odd behavior and odd speech. The total SPQ score was obtained by summing the scores from all of the items. The three schizotypal trait factors, cognitive/perceptual, interpersonal and disorganization, were derived by summing the related subscale raw scores according to the three-factor model of Raine and colleagues [32]. Full-scale IQ was assessed using the Wechsler Adult Intelligence Scale, Revised or Third edition.

Statistical analysis

Differences in clinical characteristics between the patients and the controls or between the genotype groups were analyzed using the χ^2 test for categorical variables and the Mann-Whitney *U*-test for continuous variables, using the PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS [41]). The power estimates were based on the allele frequency of 0.35 (rs2298599) in the controls and an alpha level of 0.05. Power was calculated under a prevalence of 0.01 using a multiplicative model that assumed varying degrees of the odds ratio (OR). Statistical analyses for the genetic associations were performed using the SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan). Deviation from the HWE was tested using χ^2 tests for goodness of

Table 1. Genotypic and allelic distributions for SNPs in the *p250GAP* between patients with schizophrenia and controls.

Marker				SCZ ($n=431$)			CON ($n=572$)			Genotypic	SCZ	CON	Allelic	OR
SNP IDs	Position[a]	M/m	gene	M/M	M/m	m/m	M/M	M/m	m/m	p ($\chi 2$)	MAF		p ($\chi 2$)	(95% CI)
rs493172	128388089	C/G	intron1	346	77	3	451	116	3	0.63 (0.9)	0.10	0.11	0.49 (0.5)	0.90 (0.67–1.21)
rs10893947	128375634	G/A	intron1	122	217	88	177	294	94	0.25 (2.8)	0.46	0.43	0.14 (2.2)	1.15 (0.96–1.37)
rs2276027	128355514	T/C	intron8	241	158	27	303	229	36	0.57 (1.1)	0.25	0.27	0.42 (0.7)	0.92 (0.75–1.13)
rs3796668	128349062	A/C	intron11	186	182	62	206	292	72	**0.020 (7.8)**	0.36	0.38	0.22 (1.5)	0.89 (0.74–1.07)
rs581258	128348083	A/G	exon12	293	125	8	373	171	17	0.46 (1.6)	0.17	0.18	0.32 (1.0)	0.89 (0.70–1.12)
rs3740829	128344366	A/G	exon13	375	50	2	513	54	1	0.37 (2.0)	0.06	0.05	0.18 (1.8)	1.30 (0.89–1.91)
rs546239	128340968	A/G	3′	325	91	9	402	149	12	0.18 (3.4)	0.13	0.15	0.11 (2.6)	0.81 (0.63–1.05)
rs2298599	128340162	G/A	3′	167	184	76	219	296	53	**0.00015 (17.6)**	0.39	0.35	0.07 (3.3)	1.18 (0.99–1.42)

SCZ: patients with schizophrenia, CON: controls, M: major allele, m: minor allele, MAF: minor allele frequency, OR: odds ratio, 95%CI: 95% confidence interval.
[a]db SNP build 129.
All of the alleles are represented according to the minus strand DNA sequence. Numbers of genotypes were represented as genotype counts. *P* values<0.05 are in boldface and underlined.
doi:10.1371/journal.pone.0035696.t001

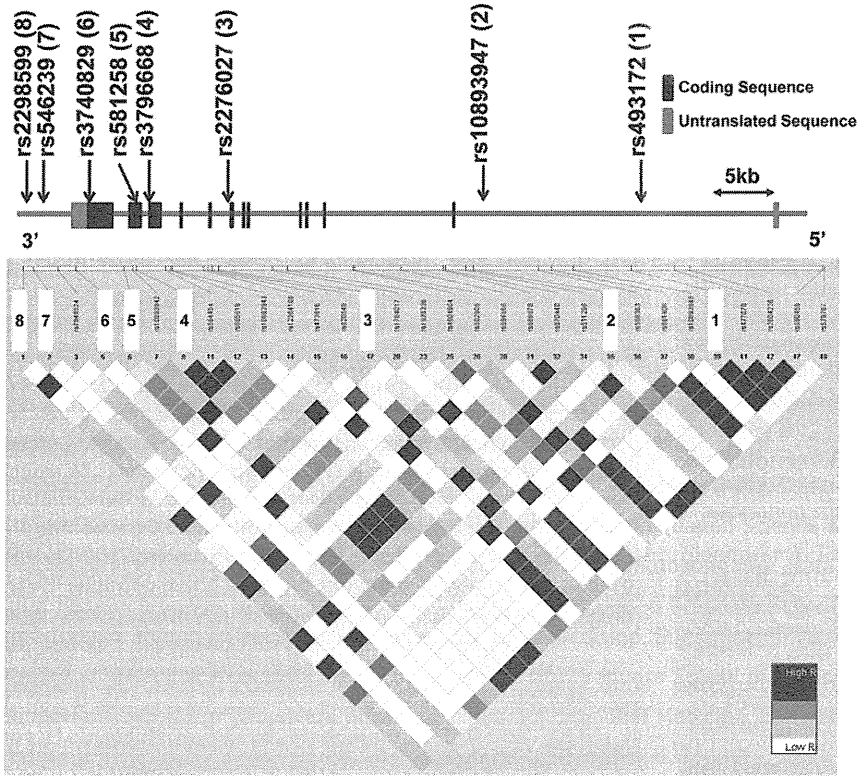

Figure 1. The genomic structure of *p250GAP* and linkage disequilibrium of the *p250GAP* in the HapMap JPT. The genomic structure of *p250GAP* is based on an entry in the Entrez Gene database (National Center for Biotechnology Information). The locations of the SNPs analyzed in this study are indicated by arrows. The numbers indicated in parentheses refer to the numbering of the SNPs in the linkage disequilibrium (LD) diagram. The distances of the exons-introns and the intermarkers are drawn to scale. The LDs between the pairwise SNPs are shown using the r^2 value at the bottom of the map of the gene structure for the HapMap JPT samples. High levels of LD are represented by black (r^2) coloring, with increasing color intensity shown by the color bars.
doi:10.1371/journal.pone.0035696.g001

fit. The allelic and genotypic distributions of *p250GAP* polymorphisms between the patients and the controls were analyzed using χ^2 tests.

Pairwise linkage disequilibrium (LD) analyses, expressed by r^2, were applied to detect the intermarker relationships in each group using the Haploview 4.2 software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype frequencies were estimated using the maximum likelihood method with the genotyping data. We used the expectation-maximization algorithm from the SNPAlyze V5.1.1 Pro software. Rare haplotypes, detected in less than 3% of the patients and the controls, were excluded from the haplotypic association analysis, as previously described [42,43]. Using a 2×2 contingency table approach, we performed 10,000 permutations of significance tests to determine empirical significance. We used a 2- to 8-window fashion analysis. We applied Bonferroni corrections in allelic and genotypic association analyses (eight tests) and in haplotypic association analyses (28 independent global tests).

The effects of the *p250GAP* genotype on the total score and on the three factors of the SPQ were analyzed by a one-way analysis of variance (ANOVA). To control confounding factors, the effect of the *p250GAP* genotype on the significance factor of the SPQ was analyzed by a one-way analysis of covariance (ANCOVA). Age, sex and education years were used as covariates because the SPQ total score and the three factors were correlated with these confounding factors in a previous study [44]. Standardized effect sizes were calculated using Cohen's *d* method (http://www.uccs.edu/faculty/lbecker). All *p* values are two tailed, and statistical significance was defined as $p<0.05$.

Results

Genetic association analysis

Our study size of 431 patients with schizophrenia and 572 controls had sufficient power (>80%) to detect a genetic effect at ORs of 1.30 or larger when the allele frequency was 0.35. The genotype and allele frequencies of the eight tagging SNPs located in the *p250GAP* gene and flanking regions are summarized in Table 1. We found significant differences in genotype frequencies between the patients and the controls in rs3796668 ($\chi^2=7.8$, $p=0.020$) and rs2298599 ($\chi^2=17.6$, $p=0.00015$). No allelic or genotypic associations were observed with schizophrenia for any other SNPs ($p>0.05$). The major genotype frequency of rs3796668 was significantly higher in the patients with schizophrenia (43%) than in the controls (36%) ($\chi^2=5.2$, $p=0.023$), but no differences were observed in the frequencies of the minor or heterozygous genotypes of rs3796668 ($p>0.05$). The minor genotype frequency of rs2298599 was higher in the patients with schizophrenia (18%) than in the controls (9%) ($\chi^2=15.5$, $p=0.000083$), but no differences were observed in the frequencies of the major or heterozygous genotypes of rs2298599 ($p>0.05$). The evidence for genotypic association of rs2298599 remained significant after a Bonferroni correction for multiple tests (corrected $p=0.0012$). Genomic sequencing data for rs2298599 for each individual were in agreement with genotyping data using the TaqMan methods. Haplotype analysis showed a marginally significant association with schizophrenia in the rs3740829- rs546239- rs2298599 haplotype ($\chi^2=7.9$, global $p=0.049$) (Table S2). However, the

Table 2. Demographic variables for subjects included in the SPQ analysis.

Variables	Total ($n=180$)	G carrier ($n=159$)	A/A ($n=21$)	p values	(z)
Age (years)	36.6±11.5	36.5±11.5	37.5±11.8	0.69	0.40
Sex (male/female)[a]	87/93	77/82	10/11	0.94	<0.01
Education (years)	15.4±2.4	15.6±2.4	14.4±2.0	**0.041**	−2.05
Full scale IQ	109.0±12.0	109.0±12.2	108.8±11.2	0.75	−0.33

Means ± SD are shown. P values<0.05 are in boldface and underlined.
[a]χ^2 test.
doi:10.1371/journal.pone.0035696.t002

association did not survive correction for multiple testing ($p>0.05$ after Bonferroni correction).

The LD relationships between the investigated markers are provided in Figure S1. The LD pattern observed in our controls was similar to our patients and the JPT HapMap samples; however, it was different from the LD pattern of the Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) HapMap samples.

In silico genotype-expression analysis

We examined an association between the rs2298599 and the expression levels of the *p250GAP* gene in immortalized lymphoblasts derived from 45 HapMap JPT subjects using WGAViewer software (http://compute1.lsrc.duke.edu/softwares/WGAViewer). However, *in silico* analysis revealed that there was no significant association between the SNP and the *p250GAP* expression in the immortalized lymphoblasts ($p=0.28$).

Impact of *p250GAP* genotype on schizotypal personality traits

We examined a possible association between the *p250GAP* genotype of rs2298599 and schizotypal personality traits in healthy subjects. Compared to controls, patients with schizophrenia were significantly more likely to carry the rs2298599 A/A genotype. Therefore, these analyses focused on a comparison of homozygous risk A/A genotype carriers versus homozygous carriers of one or two copies of the G allele (a combined G/G and G/A genotype group), under a recessive inheritance model of the risk A/A genotype. Demographic variables, age, sex, and full-scale IQ were not significantly different between the genotype groups, except for years of education ($z=-2.05$, $p=0.041$) (Table 2). We first examined the possible effect of *p250GAP* rs2298599 on the total SPQ score and found a significant effect of the genotype ($F_{1,178}=4.08$, $p=0.045$) (Table 3). Then, we investigated the genotype effects on the three SPQ factors, cognitive/perceptual, interpersonal and disorganization. A significant genotype effect was observed on the interpersonal factor ($F_{1,178}=5.85$, $p=0.017$), but no significant genotype effects were observed on the cognitive/perceptual or disorganization factors ($p>0.1$). The effect of genotype on the interpersonal factor remained significant after adjusting for confounding factors ($F_{1,175}=4.71$, $p=0.031$). Subjects with the risk A/A genotype of rs2298599 showed higher scores on schizotypal traits, particularly the interpersonal factor, compared with subjects with the G allele (Figure 2). The effect sizes of the total score and interpersonal factor were 0.41 and 0.47, respectively. When the two genotypes were divided into opposite two genotype groups (homozygous carriers of one or two copies of the A allele versus homozygous G/G genotype carriers) under a dominant model of inheritance, there was no significant difference in scores between A carriers and individuals with G/G genotype ($p>0.05$, Table S3).

Discussion

This study is the first investigation of the association of the *p250GAP* gene with schizophrenia. In this study, we first provided evidence that a genetic variant of the *p250GAP* gene was associated with the risk for schizophrenia. The frequency of individuals with the rs2298599 risk A/A genotype was higher in patients with schizophrenia than in the controls. Second, we indicated that the risk genotype of the *p250GAP* gene was associated with high schizotypal personality traits, particularly the interpersonal factor, in healthy subjects. Individuals with the rs2298599 risk A/A genotype scored higher on schizotypal personality traits and the interpersonal factor than did individuals with non-risk genotypes. These findings suggest that the *p250GAP* gene may be related to the risk for schizophrenia and the schizotypal personality traits.

Rs2298599 is situated within the relatively large LD block, which includes the *p250GAP* and the *P53AIP1* (OMIM 605426) genes (Figure S2). The SNP is located 2.9 kb downstream of the *p250GAP* gene and located 22.1 kb upstream of the *P53AIP1* gene. To confirm whether a significant association signal of rs2298599 with schizophrenia is attributed to the *p250GAP* gene, we checked strength of LDs in the genomic region (±50 kb) around rs2298599 using HapMap data (JPT, Chr 11: 128,290,162..128,390,161). Seven SNPs was related to rs2298599 with the criteria of r^2 greater than 0.8. Of the seven SNPs, five SNPs were located 5′ upstream from rs2298599 and four SNPs were included in the *p250GAP* gene. Two SNPs were located 5.2 and 7.9 kb downstream from rs2298599. These findings suggest that our association signal could be attributed to the *p250GAP* but not the *P53AIP1* gene. However, the *P53AIP1* may be a susceptibility gene for schizophrenia. Future

Table 3. Association of the *p250GAP* gene risk variant with the schizotypal personality traits.

SPQ Variables	Total ($n=180$)	G carrier ($n=159$)	A/A ($n=21$)	Cohen's d	Genotype effect $F_{1,178}$	p values	η^2
Total score	10.7±8.9	10.3±8.4	14.4±11.4	−0.41	4.08	**0.045**	0.02
Cognitive/perceptual	3.3±3.8	3.2±3.7	4.0±4.5	−0.19	0.94	0.33	0.01
Interpersonal	5.0±4.5	4.7±4.1	7.2±6.3	−0.47	5.85	**0.017**	0.03
Disorganization	3.1±3.3	3.0±3.3	4.1±3.8	−0.31	2.13	0.15	0.01

SPQ: Schizotypal Personality Questionnaire. Means ± SD are shown. The effect sizes are typically categorized as small ($d=0.20$, $\eta^2=0.01$), medium ($d=0.50$, $\eta^2=0.06$) or large ($d=0.80$, $\eta^2=0.14$). Significant p values are shown in boldface and underlined.
doi:10.1371/journal.pone.0035696.t003

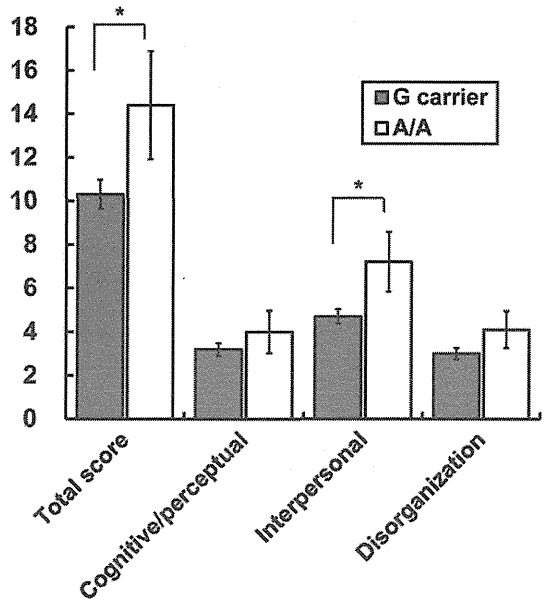

Figure 2. The association between the risk-associated *p250GAP* genotype and SPQ total score and the three factors. The gray bars represent individuals who are G-carriers (G/G and G/A genotypes) of rs2298599. The white bars represent individuals with the A/A genotype of the SNP. Error bars represent standard errors of the mean. * $p<0.05$.
doi:10.1371/journal.pone.0035696.g002

studies are required to investigate the association between the *P53AIP1* and schizophrenia.

Although significant associations between the *p250GAP* gene and schizophrenia were observed in this study, no experimental evidence has indicated that the rs2298599 SNP of *p250GAP* is functional. To define a possibly functional SNP associated with the disease, followed by evaluation of altered function caused by the relevant SNP, may be able to narrow down the region of the association observed with the rs2298599. For example, gene expression analyses at either mRNA or protein levels of the *p250GAP* gene using postmortem or lymphoblast samples may be an alternative approach. We examined an association between the rs2298599 and *p250GAP* expression in immortalized lymphoblasts. However, *in silico* analysis revealed that the SNP might not be related to *p250GAP* mRNA expression in a Japanese population. A future biological study of the function of rs2298599 or *p250GAP* gene is required to verify our results.

MicroRNAs (miRs) regulate cellular fate by controlling the stability or translation of the mRNA transcripts. The miR132, located on 17p12.3, controls p250GAP protein levels and regulates neuronal morphogenesis by decreasing the levels of p250GAP [45]. The miR132 target sequence in the *p250GAP* 3′UTR, AACAGTCCACTGTCCAGCAGAGG, is conserved across vertebrate evolution. We performed a mutation search of the genomic region (the target sequence of miR132±250 bp) in the *p250GAP* gene using 48 patients with schizophrenia to evaluate the presence of a genetic variant in this region. However, we had no polymorphisms in our sequence data. This result suggests that the miR132 target sequence in the *p250GAP* might not play a major role in risk for schizophrenia.

Several molecular genetic studies have investigated the influences of susceptibility genes for schizophrenia on schizotypal personality traits. These studies have reported associations between the *COMT* [46,47,48], *NRG1* [49], *DTNBP1* [50,51], *RGS4* [52], *DAAO* [51] and *ZNF804A* [35] genes and schizotypal components. Risk alleles or haplotypes of schizophrenia were correlated with high scores on schizotypal personality traits. Of these genes, the *COMT*, *NRG1*, *DTNBP1*, *DAAO* and *RGS4* genes, as well as the *p250GAP* gene, are directly or indirectly responsible for NMDA receptor-mediated glutamate transmission or signaling via glutamate receptors [53]. However, involvement of the glutamate NMDA receptors in SPD is still unknown. Further research will need to clarify the relationship between the glutamate NMDA receptors and SPD.

The interpretation of our results has several limitations. We found a significant association of the *p250GAP* gene with schizophrenia using 431 patients with schizophrenia and 572 controls. Our sample sizes had sufficient power (>0.80) to detect the effects of ORs of 1.30 or larger. Because our results were based on a relatively small sample to detect the effects of ORs of 1.30 or fewer, a future replication study using larger sample sizes is needed to confirm our findings. Our positive results might have been derived from a sample bias due to population stratification and non-age-matched samples, although the Japanese are a relatively homogeneous population. We used schizotypal personality traits as a phenotype of interest. As the assessment of the personality traits was based on a self-reported questionnaire, it was not an objective measurement. Importantly, to be included in the SPQ analysis, subjects were not required to meet criteria for SPD. We had hypothesized that schizotypal personality trait is a continuous measure of the genetic liability to schizophrenia. G allele carriers had marginally higher years of education and lower scores on schizotypal traits than did subjects with the risk A/A genotype. In a previous study, years of education had significant inverse effects on the total SPQ score and the three factor scores, indicating that the SPQ scores decreased with increased years of education [44]. The educational level difference between the genotype groups may have affected the genotype effects on the schizotypal personality trait. However, our results remained significant after adjusting for years of education.

In this study, we proposed *p250GAP* as a new candidate gene for susceptibility to schizophrenia. The association between the *p250GAP* gene and schizophrenia might partially explain the relationship between the hypofunction of the glutamate NMDA receptor and schizophrenia. Future studies are required to confirm the association between the *p250GAP* gene and schizophrenia in other populations.

Supporting Information

Figure S1 Linkage disequilibrium pattern of eight SNPs in the patient, control, HapMap JPT and CEU groups. The linkage disequilibriums (LDs) between the pairwise SNPs are shown using the r^2 value separately for the patients with schizophrenia, the controls, the HapMap JPT samples and the HapMap CEU samples. High levels of LD (r^2) are represented by black coloring, and increasing color intensity from 0 to 100 is shown by the color bars. The numbers (from 1 to 8) in the boxes refer to the eight tagging SNPs; rs493172 (1), rs10893947 (2), rs2276027 (3), rs3796668 (4), rs581258 (5), rs3740829 (6), rs546239 (7) and rs2298599 (8).
(TIF)

Figure S2 Linkage disequilibrium in the genomic region (±50 kb) around rs2298599 SNP in HapMap JPT. LD structure is based on an entry in the HapMap data release 24/PhaseII Nov 08, on NCBI B36 assembly, dbSNP b126 (JPT, Chr 11: 128,290,162..128,390,161). The LD structure between the pairwise SNPs is shown using the r^2 value. High levels of LD are represented by black (r^2) coloring, with increasing color intensity.
(TIF)

Table S1 Selected tagging SNPs in the *p250GAP* gene and its flanking regions.
(DOC)

Table S2 Haplotype analysis of the *p250GAP* gene between patients with schizophrenia and the controls.
(DOC)

Table S3 Association of the *p250GAP* gene variant with schizotypal personality traits under dominant model of inheritance.
(DOC)

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Author Contributions

Conceived and designed the experiments: KO RH TN TY MK MT. Performed the experiments: TO YY HY MF SU. Analyzed the data: KO RH TN MI HK. Contributed reagents/materials/analysis tools: TO YY HY MF SU MI HK TY MK MT. Wrote the paper: KO RH TN.

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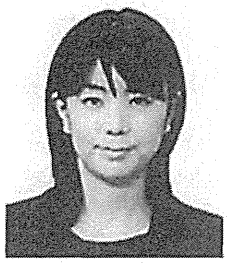
うつ病の認知行動療法
——わが国における実証的研究
Cognitive behavioral therapy for depression : Recent empirical studies in Japan



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◎認知行動療法(CBT)は，患者の認知や行動に働きかけを行う構造化された短期精神療法である．うつ病の治療として中等症以上の重症度があれば，一般的には抗うつ薬を中心とした薬物療法が行われるが，海外を中心にランダム化比較試験などのエビデンスが蓄積され，中等症以上のうつ病治療およびうつ病の再発予防に対する認知行動療法の有効性が認められている．わが国のうつ病の認知行動療法研究については，ケースコントロール研究，症例報告が中心であったが，最近はランダム化比較試験(RCT)など前向き臨床研究の報告が散見されつつある．現在のところわが国では，①不眠症状が残遺するうつ病，②閾値下うつ，③労働者のうつ病，を対象にした3つのRCTが報告されており，いずれもうつ症状の改善が認められている．今後はさらにわが国の医療体制などを加味した費用対効果に関する検討やCBTへのアクセスの観点から，CBT実施者の育成に関する研究や，コンピュータCBTの有用性に関する実証的研究なども必要となってくるであろう．

Key word：うつ病，認知行動療法(CBT)，RCT

うつ病は身体疾患と比較しても非常に多いcommon disorderのひとつである．アメリカではうつ病の地域における生涯有病率は15～17%といわれ[1]，12カ月間有病率は6～7%[2]と報告されている．一方，わが国のうつ病の有病率は，生涯有病率6.5%，12カ月間有病率2.2%[3]とアメリカの半数程度であるものの，それと関連する自殺や休職者などの増加は社会問題化している．臨床的にうつ病は，①持続的な抑うつ気分，②興味または喜びの喪失，③気力の減退，で特徴づけられる精神疾患である．精神疾患の標準診断基準であるアメリカ精神医学会が作成した精神疾患の診断・統計マニュアル(DSM-Ⅳ)[1]では，うつ病には①大うつ病性障害(major depressive disorder)：1日中持続する抑うつ気分または興味・喜びの喪失が2週間以上持続し，それに合わせて4つ以上のうつ関連症状により，著しい社会・職業などの機能障害を引き起こしている病態と，②気分変調性障害(dysthmic disorder)：大うつ病エピソードよりは症状は軽いものの，2年以上慢性的に症状が持続し社会・職業などの機能障害を引き起こしている病態，そして③躁病の病相期を認める双極性障害(いわゆる躁うつ病)のうつ病がある．

うつ病の治療として中等症以上の重症度があれば，一般的には抗うつ薬を中心とした薬物療法が行われるが，海外を中心にランダム化比較試験(randomized controlled trial：RCT)など臨床疫学的手法を用いて，認知行動療法(cognitive behavioral therapy：CBT)の有効性を示すエビデンスが蓄積されている．現在のところ単極性うつ病に対しては75以上のメタアナリシスが報告されており，中等症以上のうつ病治療およびその再発予防に対するCBTの有効性が認められている．また，うつ病治療の継続性への向上にも期待さ

れ，CBTのほうが薬物療法より早期脱落は少なく(relative risk(RR)＝0.82，95%CI：0.67-1.00)，とくに重症から極重症うつ病患者ではその有効性は顕著である(RR＝0.55，95%CI：0.32-0.94)[4]．このような背景から，アメリカやイギリスのうつ病治療ガイドライン[4,5]ではCBTの実施が推奨されている．

本稿では，うつ病治療に対するわが国におけるCBTの実証的研究について概説する．

認知行動療法(CBT)とは

CBTは，患者の認知(cognition：物事のとらえ方，解釈)や行動に働きかけを行う構造化された短期精神療法である[6]．認知的アプローチでは，うつ病患者がもつ自己・周囲・将来に対する極端に悲観的で出来事に対する解釈がうつの誘因や持続因子になっているという理解のもと，こうした悲観的で現実と乖離した思考過程を再検討することによって気分の改善をはかるものである．一方，行動的アプローチでは，うつ病患者の非適応的行動パターンに対して行動変容を促し，気分の改善をはかるものである．それらを取り組む際はコラム法，活動計画，問題解決法などCBTの各技法が用いられる．

CBTは標準的には週1回45分間，12～16回のセッションで行われる．CBTの全体構造は，①患者が抱えている問題整理を通して症例の概念化を行い，治療方針を立てる，②非機能的な悲観的思考を検証し，それに代わる柔軟で肯定的な認知の形成を促す(認知療法的アプローチ)，また患者が適応的な行動反応や問題解決を促す(行動療法的アプローチ)，③スキーマに焦点をあてる，④再発予防を含む治療終結，といった形をとる．なお，CBTでは患者自身の取り組みも重視するので，セッションとセッションの間に面接の終りにはそのセッションで話し合われたことを生かすホームワークを出すのも特徴的である．

わが国の認知行動療法研究の動向

うつ病のCBT研究についてはOnoら[7]が報告しており，1983～2009年の間に国内の文献検索データベースでは原著論文，レビュー，解説，症例報告を合わせて和文報告は485件であった．一方，海外のデータベースで検索すると，欧文報告は，MEDLINEにてケースシリーズまたはケースコントロール研究は13件，症例報告は27件であったが，2009年以降，前向き臨床研究の報告が散見されつつある．

前後比較研究

うつ病に対するマニュアルを用いた標準化されたCBTの効果研究について，Fujisawaら[8]が報告している．この研究は18～60歳でDSM-Ⅳの大うつ病性障害の診断基準を満たす者に対して毎週または隔週にわたり全16回の個人CBTプログラムを行い，その実施前後で症状評価したものである．症状評価は抑うつ症状(自覚的評価)をBeck Depression Inventory(BDI-Ⅱ)，抑うつ症状(他覚的評価)をHAMD-17，非機能的思考態度をDAS-24，全般的機能をGAF，主観的ウェルビーイングをSUBI-health scale/SUBI-fatigue scaleにて行った．研究には27名が参加し，26名がプログラムを終えた．CBT実施前後でBDI-Ⅱは32.6から11.7へ，HAMD-17は24.3から8.1と改善し，その他の評価項目についても有意な改善が認められた．

一方，Matsunagaら[9]は2剤以上の薬物療法に治療抵抗性を示したうつ病患者に対して薬物療法に並行して12週間の集団CBTを行い，うつ症状，社会機能の改善の評価を行い，実施12カ月後にその持続効果に関する評価を行った．集団CBTは1グループ当り5～6名で構成され，週1回90分のセッションを12回実施した．研究には43名がプログラムに参加し，38名が集団CBTプログラムを終えた．GAF(機能の全般的評価)，SF-36(健康関連のQOL評価)，HAMD-17(抑うつ症状の他覚的重症度)，DAS(非機能的思考態度評価)，ATQ-R(自動思考尺度)を用いて評価を行った．プログラム実施後にHAMD-17は14.7から9.2(寛解率：49%)，GAFは59.5から65.5と改善し，そのほか各評価項目は有意に改善し，1年後もその効果は継続していたことを認めた．また，田島ら[10]はうつ病の休職者に対して集団CBTを行い，抑うつ気分の改善に有効であったと報告している．

表1　わが国のうつ病・うつを対象とした認知行動療法のランダム比較試験(2012年6月現在)

研究	対象	対照	n (CBT vs. control)	期間	認知行動療法の内容	結果
Kojima (2009)	会社員	Waiting-list	137 vs. 124	3カ月間	3時間集団CBT+3回電子メールフォローアップ	両群でCES-Dにおいてうつ症状は2.33点差を認めた($p<0.01$)
Watanabe (2011)	DSM-Ⅳ大うつ病性障害，HAMDの不眠項目(すくなくとも1つ)≧2，insomnia severity index≧8	通常療法	20 vs. 17	介入期間4週，8週の観察期間	1回1時間の個人睡眠CBTセッションを毎週計4回通常療法に並行して行う	不眠の寛解：NNT=2 うつ病の寛解：NNT=2
Furukawa (2012)	うつ病の診断を有しないもののうつ症状(BDI≧10，K6≧9)を呈する会社員	既存の従業員支援プログラム	58 vs. 60	介入期間8週，4カ月間の観察期間	電話によるCBTを従業員支援プログラムに併用実施．tCBTとは，トレーニングを受けた心理療法士がマニュアル化されたCBTプログラムを全8回にわたって行う	うつ症状の改善 effect size=0.69 presenteeismの改善 effect size=0.15

ランダム化比較試験(表1)

わが国では不眠症状が残遺するうつ病，閾値下うつ，労働者を対象にしたRCTが報告されている．

Watanabeら[11]は，20～70歳でDSM-Ⅳの大うつ病性障害の診断基準を満たし，HAMDの3つある不眠項目のうち2点以上を1項目以上認め，不眠の重症度Insomnia Severity Index(ISI)が8点以上の者に対して，通常治療に並行して4週間の短期睡眠CBTを実施した場合，通常治療を比較対照としてその効果について8週間検討した．短期睡眠CBTにおける不眠症に対するCBTは，1回1時間の個人セッションを4週間にわたって行うものである．その内容は，睡眠衛生に関する教育や行動的アプローチから構成される．評価はISI(不眠の重症度)，睡眠パラメータ〔睡眠効率，総睡眠時間，睡眠潜時，中途覚醒時間をピッツバーグ睡眠質問票(PSQI)にて収集〕，GRID-HAMD(抑うつ症状の他覚的重症度)を用いた．この研究には37名が参加し，平均年齢が50.5歳，女性が62.2%であり，短期睡眠CBTに割り付けられた20名のうち19名が完遂した．その結果，短期睡眠CBTを行った群におけるISIのベースライン評価時：15.3，4週：10.6，8週：9.2であり，通常療法群はベースライン評価時：17.4，4週：15.9，8週：15.9であった．一方，HAMD-17の短期睡眠CBTを行った群におけるベースライン評価時：15.0，4週：9.9，8週：11.3に対して，通常療法群はベースライン評価時：16.8，4週：17.8，8週：18.4であった．このように，不眠症状のみならず全般的なうつ症状の改善も有意に認めたのが特徴である．

Furukawaら[12]は，うつ病の診断を有しないもののうつ症状(BDI≧10，K6≧9)を呈する会社員に対して電話によるCBT(tCBT)を従業員支援プログラム(employee assistance program：EAP)に併用実施し，既存のEAPとその有効性について検討した．tCBTとはトレーニングを受けた心理療法士がマニュアル化されたCBTプログラムを全8回にわたって行うものである．118名の会社員を対象に，EAP群(60名)とEAP+tCBT併用群(58名)に割り付け，毎週8週間にわたって介入し，抑うつ症状(自覚的評価)をBDI-Ⅱ，：労働生産性(労働効率が低下している状態：presenteeism)をHPQ(Health Performance Questionnaire)などによって評価を行った．その結果，EAP+tCBT併用群のベースライン評価時17.7が

4カ月時11.0，EAP群のベースライン評価時16.8が4カ月時15.7と有意にEAP＋tCBT併用群は改善し，その効果量は0.69であった．一方，presenteeismは，EAP＋tCBT併用群のベースライン評価時55.2が4カ月時62.4，EAP群のベースライン評価時56.3が4カ月時59.9と両群での差は認められず，presenteeismに関する効果ははっきりしなかった．

Kojimaら[13]は，261名の会社員に対して3時間の集団CBT教育プログラムと3回の電子メールフォローアップセッションのプログラムを行い，waiting-listを対照群として，うつ症状評価をCES-Dにて3カ月間行った．この研究の介入群は114名(83%)が完遂し，ベースラインから3カ月評価時点のCES-Dは，介入群では2.21点改善したのに対して対照群は0.12点悪化したことが認められた．

おわりに

うつ病に対するCBTの有効性について，それを支持する国内エビデンスがすこしずつ集積しつつある．今後はさらにわが国の医療体制などを加味し，費用対効果に関する検討やCBT実施者の育成に関する研究が必要である．また，イギリスではコンピュータによるCBT(computerized cognitive behavioral therapy：CCBT)の効果が示され，治療終了後2カ月での寛解率は従来型の対面式CBTと差がないという報告もあり(RR＝1.33, 95%CI：0.38-4.72)[4]，普及(dissemination)やCBTへのアクセスの観点からCCBTの研究も必要となってくるであろう．

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シリーズ
薬剤師に必要な精神疾患の基礎知識

第2回　大うつ病性障害

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はじめに

うつ病は，身体疾患と比較しても頻度の高いcommon disorderの1つである。疫学調査から女性は，男性より2倍程度うつ病の有病率が高く，うつ病の地域における生涯有病率は，アメリカにおいては15～17％[1]，12ヵ月間有病率は6～7％[2]と報告されている。一方，わが国のうつ病の有病率は生涯有病率6.5％，12ヵ月間有病率2.2％[3]と報告されアメリカの半数程度である。うつ病に罹患した患者のうち，約半数は10年以内に新たなエピソードを発症するといった慢性的な経過をたどることが多く[4]，また，うつ病は自殺の大きなリスク因子でもある[5]。このように有病率の高いうつ病は個人のみならず，社会や経済など様々な面に影響を与え，例えば休職者や医療サービスの利用を増大させるといわれている[6]。アメリカでは，うつ病よる国家レベルでの経済的負担は2000年では830億ドルを超えたと見積もられ，そのうち260億ドルは直接の治療コスト，50億ドルは自殺に関連したコスト，520億ドルは職域でのコストと推定されている[7]。これらの推定コストは，疾患者自身の負担，家族や介護者の負担，労働力を失ったことによる損失，無治療者に関連した費用等を含んでおらず，経済的負担はもっと大きくなるとも指摘されている。

わが国においても，うつ病と関連する自殺や休職者などの増加もあって，うつ病対策は喫緊の公衆衛生学的な課題となっている。また一方，執着性格やメランコリー親和型性格の中高年者に比較的多く発症するという古典的うつ病や，依存性が強く未熟なパーソナリティー傾向をもつうつ病など，昨今のうつ病概念の広がりからわが国の臨床現場でのうつ病の診断をめぐる混乱がみられることも少なくない。このような背景から，本稿ではアメリカ精神医学会が作成した精神疾患分類に定義されるうつ病を中心に概説し，また症例を呈示しながら服薬指導のポイントについて述べる。

大うつ病性障害と認知行動モデル

臨床的には，うつ病は持続的な抑うつ気分，興味または喜びの喪失，気力の減退で特徴づけられる精神疾患である。精神疾患の標準診断基準であるアメリカ精神医学会が作成した精神疾患の診断・統計マニュアル（以下，DSM-Ⅳ）[1]では，うつ病は(1)大うつ病性障害（major depressive disorder）（表）：2週間以上の1日中持続する抑

表　DSM-Ⅳによる大うつ病性障害（単一エピソード）の診断基準

〈文献1）より引用転載〉

A．単一の大うつ病エピソードの存在
　a．以下の症状のうち5つ（またはそれ以上）が同じ2週間の間に存在し，病前の機能からの変化を起こしている。これらの症状のうち，少なくとも1つは，(1)抑うつ気分あるいは(2)興味または喜びの喪失である。
　(1) その人自身の言明（例：悲しみまたは空虚感を感じる）か，他者の観察（例：涙を流しているようにみえる）によって示される，ほとんど1日中，ほとんど毎日の抑うつ気分
　(2) ほとんど1日中，ほとんど毎日の，すべて，またはほとんどすべての活動における興味，喜びの著しい減退（その人の言明，または他者の観察によって示される）
　(3) 食事療法をしていないのに，著しい体重減少，あるいは体重増加（例：1ヵ月で体重5％以上の変化），またはほとんど毎日の食欲の減退または増加
　(4) ほとんど毎日の不眠または睡眠過多
　(5) ほとんど毎日の精神運動性の焦燥または制止（他者によって観察可能で，ただ単に落着きがないとか，のろくなったという主観的感覚ではないもの）
　(6) ほとんど毎日の疲労感または気力の減退
　(7) ほとんど毎日の無価値感，または過剰であるか不適切な罪責感（妄想的であることもある。単に自分をとがめたり，病気になったことに対する罪の意識ではない）
　(8) 思考力や集中力の減退，または決断困難がほとんど毎日認められる（その人自身の言明による，または他者によって観察される）
　(9) 死についての反復思考（死の恐怖だけではない），特別な計画はないが反復的な自殺念慮，または自殺企図，または自殺するためのはっきりとした計画
　b．症状は混合性エピソードの基準を満たさない
　c．症状は臨床的に著しい苦痛，または社会的，職業的，またはほかの重要な領域における機能の障害を引き起こしている
　d．症状は，物質（例：薬物乱用，投薬）の直接的な生理学的作用，または一般的身体疾患（例：甲状腺機能低下症）によるものではない
　e．症状は死別反応ではうまく説明されない。すなわち，愛する者を失った後，症状が2ヵ月を超えて続くか，または著明な機能不全，無価値感への病的なとらわれ，自殺念慮，精神病性の症状，精神運動制止があることで特徴づけられる。
B．大うつ病性エピソードは失調感情障害ではうまく説明されず，統合失調症，統合失調様障害，妄想性障害，または特定不能の精神病性障害と重なってはいない。
C．躁病エピソード，混合性エピソード，軽躁病エピソードが存在したことがない。

薬物療法の導入 ーアドヒアランス向上を念頭にして

本症例では，ほとんど1日中，気分の落ち込みや自責感，無価値感，興味の喪失がみられている。また食欲の低下，不眠もみられ，休日は寝ころんで過ごすことが多いことから気力の低下，体力低下や易疲労性も認められる。さらに仕事の効率が落ちていること，仕事で決断できなくなっていることより，思考力や集中力の減退，決断困難がみられる。これらの症状が4ヵ月間持続しており，仕事にも支障が出てきていること，また職場のみならず，妻がいつもと様子が違うと考えていることより，家庭でも症状を認めていることから，職場でも，家庭でも機能障害がみられると判断できる。機能障害の程度として，仕事の効率が下がるなど，実際問題として支障が出ているが，何もできない程，機能障害が著しいわけではないことより，中等度の大うつ病性障害と診断できる。

幾つかの治療ガイドライン[6,8,9]では，中等症以上のうつ病においては，抗うつ薬の有効性が示され推奨されている。実臨床では，第1選択薬として，選択的セロトニン再取り込み阻害剤（SSRI），セロトニン・ノルアドレナリン再取り込み阻害剤（SNRI），ノルアドレナリン作動性・特異的セロトニン作動性抗うつ薬（NaSSA）などの新規抗うつ薬が使用されることが多いが，三環系抗うつ薬（以下，TCA）/non-TCAが用いられることもあるとされている。今回の症例では，「薬なんか飲んでも性格は治らないでしょうし，薬を飲むとその副作用が心配です……」と薬物療法に対する不安・不信，また薬の副作用への不安もあるため，薬物療法に対する理解を促すと同時に治療継続性の高い薬剤を選択するなどアドヒアランス向上への配慮も重要となるであろう。うつ病患者の半数は，アドヒアランスに関する深刻な問題がみられるという報告もあり[10]，アドヒアランスの向上をはかるためには，うつ病の疾患教育の効果がメタアナリシスで示されている[11]。またCiprianiら[12]のメタアナリシス研究に基づくならば，新規抗うつ薬の急性期治療における治療継続性が高いエスシタロプラムやセルトラリンが薬剤選択の候補として挙げられるだろう。

患者は，「病気ではなく怠けているだけ」「こんな性格なので治らない」「仕事上の問題だから服薬をしても仕方がない」と否定的認知も絡んで，うつ病の否認など極端な考えを抱くことも多い。このようことから，まず大切なのは，患者と医療者において適切な治療関係を築くことである。その際，医療者の態度としては，誠実さ，肯定的配慮，温かさ，適切な共感に十分に注意を払うべきである。そのうえで，患者にうつ病の症状にはどのようなものがあって，どのような治療を行っていくとどのようになっていくかの見通しなどを示しつつうつ病の疾患教育を行い，治療に対する不安への軽減に努めるべきであろう。薬剤選択をする際にも，医療者は患者の意向を聴取するなどの工夫が大切である。また，面接場面での疾患教育を補うために，パンフレットや書籍，ウェブサイトなどを紹介し，患者自らも治療に参画しているという感覚をもっていただくことも1つの工夫である。その他，アドヒアランス向上のための工夫として，薬物療法へのアドヒアランスを含む考えについて患者と定期的に話題にすること，煩雑さのないシンプルな服薬スケジュールを考案すること，副作用を評価しそれへの効果的な対処法を検討すること等，現実的な問題に取り組むことが挙げられる。

抗うつ薬の服薬指導のポイント ーコミュニケーションで注意点

服薬アドヒアランスがない場合に，服薬指導のポイントとしては，「それはよくないことだ」と否定するのではなく，その患者が自分の気持ちを伝えてきた点を大切にし，まず，服薬にかかわる問題を「ノーマライズする」ことが挙げられる。「薬を飲み忘れることは誰にでも一度や二度はあるものです」などと声掛けをすることで，患者がアドヒアランスの問題について責められることなく，医療者と話しやすい環境であるかに留意する。すなわち，服薬が不規則になっていることを患者が話しやすい関係をつくることが重要である。こうした患者が話しやすい関係をつくっておくことにより，患者との良好な関係を構築でき，患者のうつ病や薬剤に対する考え方や患者の行動パターンが把握しやすくなり，症状の悪化も防ぎやすい。

また患者の日課について質問し，規則正しい生活を送っていない場合，規則正しい生活を送るための工夫を指導することで，毎日決まった時間に服薬することを目指すのも1つの方法である。また，いつも同じ覚えやすい場所に薬剤を保管しておくことや服薬と日課を組み合わせることで，パターン化した行動と服薬を結び付け，薬物療法を日課の一部にすると，アドヒアランスが向上するともいわれている。また，ピルケースや服薬カレンダーなどのリマインダーシステムの利用も役立つであろう。アドヒアランスの妨げとなるものがあるかどうかを聞くことも重要であり，実際的な問題があれば，解決プ

うつ気分または興味・喜びの喪失と４つのうつ病の周辺症状により著しい社会・職業等の機能障害を引き起こしているものと，(2)気分変調性障害（dysthmic disorder）：大うつ病エピソードよりは症状が軽いが，慢性的に２年以上持続し社会・職業等の機能障害を引き起こしているものに分けられている。なお，DSM-Ⅳでは大うつ病性障害の臨床的重症区分として，軽症から中等症をうつ症状の存在に加えて幾つかの機能障害を認めるもの，重症をそれらの症候に加えて焦燥または精神運動制止に著しい身体症状を認めるものとしている。なお，DSM-Ⅳでは大うつ病性障害の診断基準は満たさないものの，はっきりと確認できるストレス因子に反応して，そのストレス因子の始まりから３ヵ月以内に情緒面または行動面に顕著な症状が出現（そのストレス因子に曝露されたときに予想されるものをはるかに超えた苦痛または，社会的または職業的・学業上の機能に著しい障害を来す）を認めるものを適応障害（adjustment disorder）という。

上記の疾患を有する者は，「認知」，「気分」，「行動」，「身体」と多岐の面にわたって影響が認められている。「認知」とは，物事に対する考え方や解釈の仕方であるが，大うつ病性障害患者の場合，自分・周囲・将来に対して非現実的で極端な（多くの場合悲観的）解釈（「否定的認知の３徴」とも呼ばれる）を認めることが多い。自分自身に対する否定的な認知としては，「自分は駄目な人間だ」，「自分には何の力もない」という例が挙げられる。周囲に対する否定的な認知というのは，周囲との対人関係において悲観的になり，「周囲の人たちは，自分のことを迷惑だと考えている」，「周囲の人たちから嫌われ，助けてくれない」等というものであり，将来に対する否定的な認知とは，将来に対して絶望的になってしまい，「これからもつらいことばかりだ」，「この先，良いことなんか起こるはずがない」，「今後も何をしても変わらない」というものがある。こうした物事への考えは気分や行動に影響を与え，気分であれば悲しみや不安を生じ，行動としては先延ばしや回避，閉じこもりなどのパターンを呈するようになることがある。そして，先延ばしという回避行動パターンを取った結果，「やっぱり自分は駄目な人間だ」という考えがますます強まり，悲しみが強くなり，さらに他者と交流をとらなくなり引きこもるという悪循環を呈するようになるのである（うつ病の認知行動モデル：図）。うつ病の患者に服薬指導や疾患教育をする際には，このような認知行動パターンに陥っていることを十分に認識すべきである。

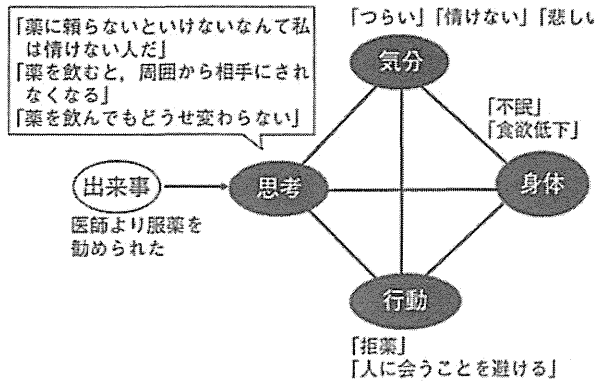

図　うつ病の認知行動モデル

症例検討

症例

大うつ病性障害の男性(35歳)。同胞なし。大学卒業後，現在の会社に就職した。元来より真面目で仕事熱心であり，仕事も丁寧だということで社内の評価も高かった。X年４月，課長に出世し，業務量も係長の時と比較して増え，また仕事内容で判断を求められる場面が増えるようになった。毎晩残業することで対処していったが，次第に仕事が溜まりがちとなった。X年７月頃より，自分は課長なのだから仕事をきちんとこなしていかなければならないと考え，できない自分に対して，「自分は課長失格だ」，「なんて自分は駄目な人間なんだ」と自分を責めるようになった。家でも，仕事のことが頭から離れなくなり，不眠や食欲低下を認めるようになった。気分も落ち込むようになり，以前はカメラが趣味で休日は良く写真を撮りに出かけていたが，趣味のカメラにも興味がなくなり，休日は家で寝ころんで過ごすようになった。仕事の効率がますます落ち，頭が回らないと感じるようになり，体力もなくなり疲れやすくなり，仕事で判断を求められても，決断できなくなった。X年８月，今までと様子が違う夫を心配した妻と一緒に精神科を受診した。

診察時，「私が仕事をきちんとできないから悪いんです。……会社の人にも，大変な迷惑をかけてしまっています。これは，私が課長の器ではないから起きたことであって……，病気っていうわけじゃないと思います。私は，もともとこういう人間なんです，弱いんです……。薬なんか飲んでも性格は治らないでしょうし，薬を飲むとその副作用が心配です……。この状況から，逃げ出してしまいたいとも思うのですが，家族のことを考えるとそれもできず……。死んでしまいたいとまでは思わないのですが……」と，ぼそぼそ小さい声で話す。

ランをたてることで，修正することが可能となるであろう。例えば，外出した時に，昼食後の薬を服用することを忘れる場合は，外出する際にもっていく鞄に予備の薬を入れておくなどの工夫により，アドヒアランスの低下を防ぐことができる可能性がある。

また，患者によっては，症状に付与する意味付けを行い，例えば「ストレスが溜まっているだけだ。治療を要する病気ではない」という病気に対する否認がアドヒアランスの低下につながっていることもよくみうけられる。アドヒアランスが低下している患者に典型的な薬物療法に対する否定的な思考としては「抗うつ薬を飲むのは私が弱い人間だからだ」「私は必ず副作用に悩まされる人間だ。もし，薬に副作用があるなら，私はそれに悩まされるだろう」「いったん，抗うつ薬を服用すると，薬に依存してしまうようになる」「薬を服用すると，自分が自分でなくなってしまう」といったものが挙げられる。これらの思考に対して，それに当てはまる事実や見落としている（またはその考えにそぐわない）事実がないかを検討することや，服薬すると，自分にとってどんなメリットがあるのかを検討すると良い。例えば，「服薬することによってどのような問題を解決でき，また防ぐことができるのか」，「仕事・対人関係・その他の活動において，薬がどのように改善するのか」，などを検討することにより，服薬に対するポジティブな面を患者と話せるようになることが有用となっていくであろう。本稿で示した症例では，服薬に対する否定的な考えがあるため，まず疾患教育を実施し，その際得られた情報を基に，医療者は患者と薬物療法を行うことのメリットとデメリットを検討し，服薬に対する思考の幅を広げ，最終的には薬物療法を納得してもらうことで，アドヒアランスの向上をはかっていくことが期待される。

おわりに

うつ病に対する薬物療法においては，再発予防を含めると長期に及ぶ治療を継続することが必要な場合が多い。このようなことから，患者と薬物療法を検討する際，(1)患者の薬物療法への理解不足や服薬に対する考え（「周囲が服薬を続けていることはだめな人間と考えている」），(2)副作用のモニタリング，(3)服薬パターン/生活スタイルのチェック，などのポイントがある。こうした患者の薬物療法への気持ちを把握することは，治療を行ううえで極めて重要である。エビデンスに基づく医療（evidence-based medicine：以下，EBM）では，質の高いエビデンスと患者自身の価値観を合わせて判断すること（いわゆるshared decision making）がEBM実践において重要であることは強調されている。こうしたEBM実践の観点からも患者の声に耳を傾け，患者とともに治療について相談していく姿勢が大切となる。

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