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Novel rare variants in F-box protein 45 (*FBXO45*) in schizophrenia

Chenyao Wang [a], Takayoshi Koide [a], Hiroki Kimura [a], Shohko Kunimoto [a], Akira Yoshimi [a], Yukako Nakamura [a], Itaru Kushima [a], Masahiro Banno [a], Naoko Kawano [a], Yuto Takasaki [a], Jingrui Xing [a], Yukihiro Noda [d], Akihiro Mouri [d], Branko Aleksic [a,*], Masashi Ikeda [b], Takashi Okada [a], Tetsuya Iidaka [a], Toshiya Inada [c], Nakao Iwata [b], Norio Ozaki [a]

[a] *Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[b] *Department of Psychiatry, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan*
[c] *Institute of Neuropsychiatry, Seiwa Hospital, Tokyo, Japan*
[d] *Division of Clinical Sciences and Neuropsychopharmacology, Graduate School of Pharmacy, Meijo University, Nagoya, Japan*

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ABSTRACT

The ubiquitin ligase F-box protein 45 (FBXO45) is critical for synaptogenesis, neuronal migration, and synaptic transmission. *FBXO45* is included in the 3q29 microdeletion region that confers a significant risk for schizophrenia, as shown by rare structural variant studies. Thus, *FBXO45* is considered a prominent candidate for mediating schizophrenia pathogenesis. Here, we investigated rare, deleterious single nucleotide variants (SNVs) as well as small insertions and deletions (INDELs) in *FBXO45* that may contribute to schizophrenia susceptibility.

Using Sanger sequencing, we performed mutation screening in *FBXO45* exon regions in 337 schizophrenia patients. Novel missense or nonsense variants were followed up with a genetic association study in an independent sample set of 601 schizophrenia patients and 916 controls, a case report for assessing the clinical consequence of the mutations, a pedigree study for measuring mutation inheritance in the proband's family, bioinformatics analyses for evaluating mutation effect on protein structure and function, and mRNA expression analysis for examining mutation transcriptional influence on *FBXO45* expression.

One heterozygous, novel, and rare missense mutation (R108C) was identified in a single schizophrenia patient and in his healthy mother. At age 20, this patient was diagnosed with paranoid schizophrenia and carried some clinical features of 3q29 deletion phenotypes, including premorbid IQ decline. With follow-up genotyping, this mutation was not found in either the schizophrenia group (0/601) or the healthy control group (0/916). Bioinformatics analyses predicted that R108C probably pathologically impacted the structure and function of the FBXO45 protein. The relative expression of *FBXO45* in SCZ case with R108C mutation was relatively low when compared to 50 schizophrenia patients and 52 healthy controls.

The R108C mutation in *FBXO45* is a rare variant with a modest effect on schizophrenia risk that may disrupt the structure and function of the FBXO45 protein. Our findings also suggest that *FBXO45* may be a new attractive candidate gene for schizophrenia.

1. Introduction

Schizophrenia (SCZ) is a severe psychiatric disorder with a lifetime prevalence of around 1% (Lewis and Lieberman, 2000) and a heritability of 64% (Lichtenstein et al., 2009). Despite high heritability, the genetic basis of SCZ remains largely unknown despite many years of researches. The genetic architecture of SCZ has been explored through genome-wide association studies (GWAS), rare structural variant studies, and next-generation sequencing (NGS). GWAS have identified common variants with extremely small effects on risk and have clarified that heritability of SCZ cannot be explained only by such common variants (Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009). The rare variant model is supported by rare structural variant studies in which individual rare copy number variants (CNVs) with large effects increase susceptibility to SCZ (Consortium, 2008; Stefansson et al., 2008; Walsh et al., 2008). NGS analysis suggested that some SCZ cases are caused by highly penetrant de novo variants (Girard et al., 2011; Xu et al., 2011; Need et al., 2012; Xu et al., 2012).

Several lines of evidence from rare structural variant studies have demonstrated that a 0.8- to 1.6-Mb deletion spanning 3q29 confers a significant risk for SCZ (Consortium, 2008; Walsh et al., 2008; Mulle et al., 2010; Levinson et al., 2011; Vacic et al., 2011). The clinical phenotype of the 3q29 deletion often includes mild to moderate mental retardation, autistic features, symptoms of SCZ, microcephaly, and dysmorphology (chest wall deformity, high nasal bridge, cleft palate,

* Corresponding author at: Department of Psychiatry, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Tel.: +81 52 7442282; fax: +81 52 7442293.
E-mail address: branko@med.nagoya-u.ac.jp (B. Aleksic).

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horseshoe kidney, etc.) (Willatt et al., 2005; Ballif et al., 2008). Additionally, genome-wide linkage analyses suggest a significant linkage between SCZ and bipolar disorder and the chromosome 3q29 telomere region (Bailer et al., 2002; Devlin et al., 2002; Schosser et al., 2004; Schosser et al., 2007). The commonly deleted 3q29 microdeletion region spans about 20 genes, one of which is *F-box protein 45 (FBXO45)*, which encodes an ubiquitin ligase.

Ubiquitylation is a rapid, local, and reversible post-translational modification of proteins that is related to regulation of synaptic processes (DiAntonio and Hicke, 2004; Kawabe and Brose, 2011). The correlation between SCZ and dysregulation of ubiquitin proteasome system (UPS) has been implicated by a variety of gene expression analyses in post-mortem brain tissue (Vawter et al., 2001; Middleton et al., 2002; Vawter et al., 2002; Altar et al., 2005) and peripheral blood (Bousman et al., 2010a; Bousman et al., 2010b). Protein ubiquitylation is catalyzed by a cascade of enzyme reactions that includes three classes of enzymes: E1 (ubiquitin-activating enzymes), E2 (ubiquitin-conjugating enzymes), and E3 (ubiquitin-protein ligases). The specificity of ubiquitylation is mainly determined by the E3 ligases, which transfer ubiquitin to substrate proteins (Kawabe and Brose, 2011). F-box proteins, a type of E3 ligase, are a large and diverse family of proteins present in all eukaryotes. Their activity is crucial for selecting proteins that will be targeted by E3 ligase (Bai et al., 1996). FBXO45 is a member of the F-box protein family and is required for normal synaptogenesis, axon navigation, and neuronal migration in developing central and peripheral neurons through UPS (Saiga et al., 2009). FBXO45 also negatively regulates neurotransmission in mature hippocampal neurons through ubiquitylation (Tada et al., 2010). Two proteins, FSN-1 and Fsn, which are the invertebrate homologues of FBXO45, were reported to regulate presynaptic differentiation (Liao et al., 2004) and terminal synaptic growth (Wu et al., 2007) through ubiquitylation proteolysis.

Considering that FBXO45 is included in the 3q29 microdeletion region and that the FBXO45 protein plays various roles in synaptic development and transmission via UPS, FBXO45 may be a novel candidate gene for SCZ. No common variants associated with SCZ were detected with the Japanese GWAS of SCZ (JPN_GWAS) in the region of FBXO45 (Fig. S1) (Ikeda et al., 2011) or by other SCZ GWAS (Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009). To investigate rare variants in FBXO45 that may contribute to susceptibility to SCZ, we conducted mutation screening in the exon regions of FBXO45 and performed follow-up analyses.

2. Materials and methods

2.1. Participants

Three sample groups were used in this study. The first group (resequencing sample set), comprising 337 SCZ patients (mean age 49.3 ± 14.6 years, male/female = 200/137), was used for mutation screening. The second group (genotyping sample set), included 601 SCZ patients (mean age 52.2 ± 15.0 years, male/female = 355/246) and 916 healthy comparison individuals (mean age 38.9 ± 15.5 years, male/female = 386/530), was used for a genetic association study. The third group (mRNA expression sample set), comprised 50 SCZ patients (mean age 42.5 ± 11.0 years, male/female = 24/26), 52 healthy controls (mean age 41.7 ± 11.5 years old, male/female = 25/27), one SCZ patient with a rare missense mutation (R108C; 50 years old, male) detected with resequencing analysis and his mother with same mutation (77 years old female). It was a smaller but representative (matched in age, and gender) sample set for assessment of genetic expression. The mutation screening, genetic association and mRNA expression samples were collected independently at each university hospital. The Ethics Committees of the Nagoya University Graduate School of Medicine and associated institutes and hospitals approved this study. Written informed consent was obtained from all participants. In addition, the patients' capacity to consent was confirmed by a family member when needed. Individuals with a legal measure of reduced capacity were excluded. Patients were included in the study if they (1) met DSM-IV-TR criteria for SCZ and (2) were physically healthy. A general characterization and psychiatric assessment of the participants is available elsewhere (Ikeda et al., 2011). Controls were selected from the general population and had no personal or family history of psychiatric disorders (first-degree relatives only based on the subject's interview). The selection was based on questionnaire responses from the controls themselves during the sample inclusion step and based on an unstructured diagnostic interview done by an experienced psychiatrist during the blood collection step.

2.2. Resequencing analysis

Human *FBXO45* spans approximately 20 kb on chromosome 3q29 (chr3: 196,295,559-196,315,930; human reference sequence GRCh37). Genomic DNA was extracted from whole blood or saliva using a QIAamp DNA blood kit or tissue kit (QIAGEN Ltd., Hilden, Germany). Optimal polymerase chain reaction (PCR) primer sequences were generated with FastPCR (PrimerDigital Ltd., Helsinki, Finland) (Kalendar et al., 2011) and validated with PerlPrimer (Marshall, 2004). To target *FBXO45*, we designed three amplicons to cover the coding exons. After PCR amplification, aliquots of PCR products were purified using Illustra Exonuclease I and Alkaline Phosphatase (GE Healthcare & Life Science, Little Chalfont, United Kingdom). These were then sequenced using the Sanger method and a 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Mutation Surveyor® (Softgenetics, State College, PA, USA) was used for mutation detection analysis in all *FBXO45* exons. The genetic variants were verified by re-amplifying and resequencing the fragments. Considering that one of the limitations of Sanger sequencing is that it cannot discover large structural variations, we screened for deletion or duplication within *FBXO45* or 3q29 region in our resequencing sample set using TaqMan copy number assays (detailed information are provided in supplementary method section).

2.3. Follow-up analyses

2.3.1. Prioritizing steps of genetic variants for follow-up analyses

Two prioritizing step genetic variants were conducted as follows: (1) we included only novel genetic variants. "Novel" was defined in our study as variants not registered in either dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) or the Exome Variant Server (http://evs.gs.washington.edu/EVS/) or 1000 Genomes (http://www.1000genomes.org/home), and (2) we included only nonsense or missense (functionally relevant) SNVs, splicing variants, and small (<900 base pairs) INDELs.

2.3.2. Genetic association study

First, to verify the frequency of variants detected in the resequencing procedure in SCZ cases and controls, we conducted a genetic association study with the genotyping sample set. Variants were designated as 'rare' if their minor allele frequency (MAF) in combined cases and controls was <1% (Schork et al., 2009). Genotyping was conducted with Custom TaqMan SNP genotyping assays (Applied Biosystems) and a Real-Time PCR System (7900HT Fast Real-Time PCR System, Applied Biosystems). Differences in the allele and genotype frequencies of SNPs between SCZ patients and controls were evaluated using Fisher's exact test (one-tail). The threshold of significance was set as $P < 0.05$.

2.3.3. Bioinformatics analyses

The genetic position and sequence were obtained from the Ensembl Genome Browser (Ensembl 70, Jan 2013). The potential structural and functional consequences of the missense mutation were evaluated using the following tools: (1) localization of the protein domain with the Human Protein Reference Database (http://www.hprd.org/index_html), (2) prediction and comparison of secondary and tertiary protein

structure changes with the I-TASSER algorithm (Roy et al., 2010) and UCSF Chimera (Pettersen et al., 2004), (3) prediction of qualitatively functional effects, i.e., benign/possibly damaging/probably damaging with Polyphen-2 and PMut software (Ferrer-Costa et al., 2005; Adzhubei et al., 2013), (4) sequence alignment of F-box proteins with BLAST (http://blast.ncbi.nlm.nih.gov/), and (5) evolutionary conservation with the HomoloGene database (http://www.ncbi.nlm.nih.gov/homologene/).

2.3.4. Analysis of mRNA levels by gene expression profiling

To investigate the transcriptional impact of the rare missense mutation R108C in *FBXO45*, we performed gene expression profiling of lymphoblastoid cell lines (LCLs) from the expression sample set. LCLs were established by Epstein–Barr virus transformation of lymphocytes and cultured in RPMI-1460 medium containing 20% fetal bovine serum, penicillin, and streptomycin. Total RNA was extracted from LCLs using a RNAqueous Kit (Invitrogen, Carlsbad, CA, USA), treated with DNase using a TURBO DNA-free™ Kit (Invitrogen), and reverse transcribed to cDNA with a High capacity RNA-to-cDNA Kit (Invitrogen). Two housekeeping genes, beta-2-microglobulin (*B2M*) and glucuronidase-beta (*GUSB*), were selected as internal control genes to normalize the PCR. Real-Time quantitative PCR was performed with the probes in the predesigned TaqMan Gene Expression Assay (Hs00397889_m1 for *FBXO45*, Hs99999907_ml for *B2M*, and Hs99999908_ml for *GUSB*; Applied Biosystems) using Applied Biosystems 7900HT. The expression probe for *FBXO45* was designed to bind the region which is not harboring mutation detected in the mutation screening analysis. Measurement of the cycle threshold was performed in duplicate. Data of relative expression level were analyzed with the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). The Mann–Whitney *U* test was used to compare expression levels of *FBXO45* between SCZ patients and controls because this test is robust in the case of deviation from normal distribution. $P < 0.05$ was considered significant.

3. Results

3.1. Mutations detected with resequencing analysis

We detected one missense variant (R108C) in exon 2, one synonymous (A11A) variant in exon 1, and two intronic variants (ss709035634 and ss79085393). These were all single-base heterozygous substitutions. One of the intronic variants was a known SNP (rs79085393), and the other three mutations were novel. The location of these variants in *FBXO45* is illustrated in Fig. 1a and b and summarized in Table 1. No deletions or duplications within *FBXO45* and 3q29 region were detected in our resequencing sample set using TaqMan copy number assays (supplement). Because the purpose of our research was to identify novel, functional mutations (missense and nonsense mutations), we excluded the known SNPs and the intronic and synonymous variants from our subsequent analyses. R108C, a heterozygous nucleotide substitution from cytidine to thymidine that was exclusively identified in a single patient out of 337, was studied with follow-up analyses (case study, pedigree study, genetic association study, bioinformatics analyses, and mRNA expression analysis).

3.2. Follow-up analysis of the rare missense variant R108C

3.2.1. Case report of the patient with the FBXO45 R108C mutation

The patient with the *FBXO45* R108C mutation was a male diagnosed with paranoid SCZ. The premorbid IQ score of the patient was estimated

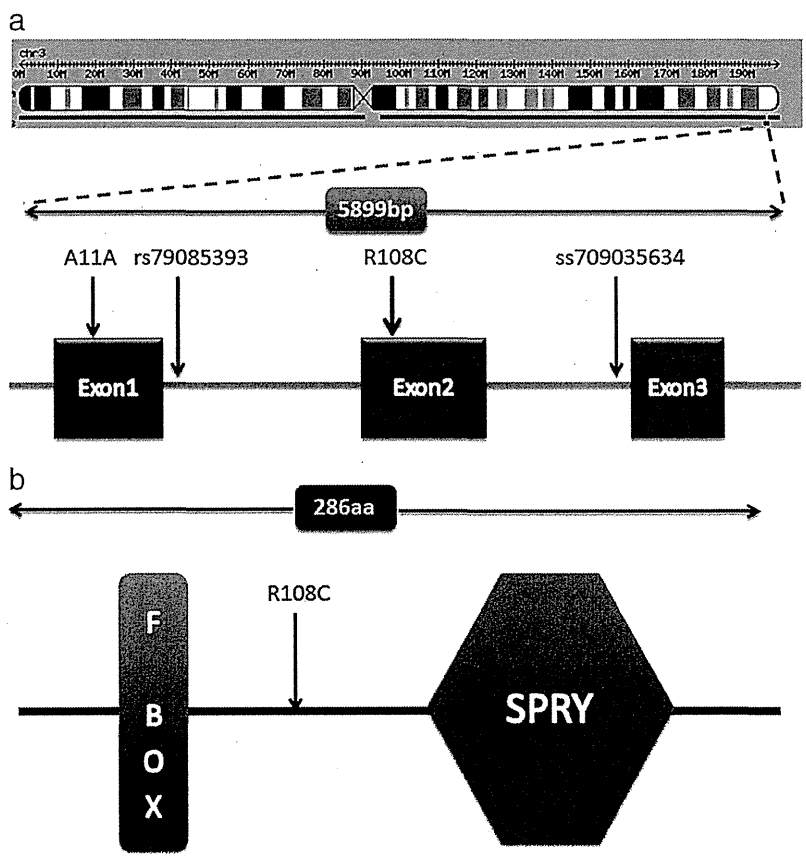

Fig. 1. Schematic genomic structure and domains of *FBXO45* and the locations of the variants studied. Legend: (a) gene structure of *FBXO45* and the locations of all the variants we detected; (b) domains of FBXO45 including the N-terminal F-BOX motif, the SPRY motif, and the location of the R108C mutation that we analyzed further.

Table 1
FBXO45 variants identified in mutation screening and association study of the missense mutation.

Chr	Genomic position[a]	Nucleotide change	dbSNP reference	Novel[b]	AA change[c]	Mutation screening[d]	Association study[d]		Combined association study[d]	
						SCZ	SCZ	CONT	SCZ	CONT
3	196295888	c.33C>T	ss709035633	Yes	A11A	0/14/323				
3	196296182	G>C	rs79085393	No	–	0/10/327				
3	196304327	c.322C>T	ss709035628	Yes	R108C	0/1/336	0/0/601	0/0/916	0/1/937	0/0/916
3	196310954	A>G	ss709035634	Yes	–	0/1/336				

[a] Genomic position based on NCBI build 37.1.
[b] No registration in either dbSNP and/or Exome Variant Server was considered as "novel".
[c] Amino acid change based on NCBI reference sequence NP_001099043.1.
[d] Genotype count: homozygote of minor allele/heterozygote/homozygote of major allele.

at 88 with the Japanese Adult Reading Test (JART), suggesting a decline of approximately 1 S.D. from the mean premorbid IQ of SCZ patients (102.2 ± 11.6) (Hori et al., 2008). We examined the cognitive performance and symptomatology of the patient with the variant (R108C) using the Positive and Negative Symptom Scale (PANSS), Brief Assessment of Cognition in Schizophrenia, Japanese Version (BACS-J) (Kaneda et al., 2007), and Continuous Performance Test, Identical Pairs version (CPT-IP) (Koide et al., 2012), the results of which are described in the supplements (Tables S1 and S2, Figs. S2 and S3). The other phenotypes of 3q29 microdeletion including autism, microcephaly, cleft palate, pectus excavatum, and horseshoe kidney were not found in this patient (Ballif et al., 2008).

3.3. Pedigree study

The mother of the proband who carried the R108C variant had no history of medical or mental illness, but the father suffered from Alzheimer's disease. The proband had no brothers, sisters, or children. DNA was obtained from the mother in whom the R108C variant was detected. The father's DNA could not be obtained due to his physical condition.

3.4. Genetic association study

The R108C variant was then searched for in the genotyping sample set and was not detected in either the 601 SCZ patients or the 916 healthy controls. Using the combined resequencing and genotyping samples, the R108C mutation was not statistically overrepresented in SCZ patients compared to controls (Table 1).

3.5. Bioinformatics analyses

Prediction of the structural effect of the R108C mutation was presented as follows. Judging the parallel between the wild-type and mutant FBXO45 protein structure predicted by the I-TASSER server, a neutral charged amino acid (cysteine) was substituted for an amino acid with a positive side chain (arginine) at codon 108, resulting in a reversed hydrophobic distribution in the alpha helix next to the SPRY domain (Fig. 2).

Prediction of the functional effect of the R108C mutation was presented as follows, two different kinds of algorithms (Polyphen-2 and PMut) both predicted that the R108C mutation will have a damaging impact on the function of the FBXO45 protein.

Conservation analysis in species was performed, and the protein and DNA sequences of *FBXO45* in different species are highly conserved from *Caenorhabditis elegans* to mammals (over 90% identical amino acids between human and mouse). R108C was located in an evolutionarily conserved region (Table 2).

Conservation analysis of F-box proteins was performed. We aligned the FBXO45 protein sequence with other F-box proteins (FBXW7, SKP2) and found that Arg108 was conserved among these F-box proteins (Table 2).

3.6. Analysis of mRNA expression

To investigate the effect of the R108C mutation on *FBXO45* mRNA expression, we separately compared the relative expression of R108C carriers with 50 SCZ patients and 52 controls. As seen with the box plot depicting data of the relative expression of *FBXO45* normalized to the two housekeeping genes, *B2M* and *GUSB*, the relative expression of *FBXO45* in the R108C case appeared to deviate markedly from the 50 SCZ patients and the 52 controls (Fig. 3). Interestingly, patient's mother (who is not suffering from schizophrenia) is R108C mutation carrier; however, her *FBXO45* expression level was not reduced (Fig. 3). The relative expression level of *FBXO45* did not show a nominally significant difference among the 50 SCZ patients and 52 controls ($P = 0.36$, directional test, with the Mann–Whitney *U* test).

4. Discussion

4.1. Main findings

To our knowledge, this is the first study that systematically screened all *FBXO45* coding regions to search for rare mutations in SCZ patients and assessed the association of an identified mutation with SCZ.

We found one heterozygous, novel, and rare missense mutation (R108C) in a single patient among 337 SCZ patients (resequencing sample set). Follow-up genotyping in an independent sample of 601 cases and 916 controls (genotyping sample set) revealed no carrier of R108C. Using the combined association study including the resequencing and genotyping sample set, no significant association between SCZ and R108C in *FBXO45* was detected ($P > 0.05$). Because the R108C mutation was a very rare variant in our sample set, we could not determine its significant association with SCZ or estimate its odds ratio. A further study with a larger sample size is needed to precisely verify the odds ratio of the R108C mutation in SCZ, and reconsider an association between mutation and disease.

We followed up the rare missense mutation R108C using a case report for assessing the clinical consequence of the mutation, a pedigree study for measuring the inheritance of the mutation in the proband's family, bioinformatics analyses for evaluating the impact of the mutation on the structure and function of the protein, and analysis of mRNA expression for examining the influence of transcription. We tried to analyze protein expression of FBXO45 in LCLs using western blotting. However, due to low enrichment of FBXO45 protein in LCLs, we were not able to detect FBXO45 in schizophrenia patients and healthy controls (supplementary information).

The patient with the *FBXO45* R108C mutation was a male who was diagnosed with the paranoid type of SCZ. Of all the symptoms, physical signs, and examination results of this patient, only the lower premorbid IQ (score = 88 with JART) appeared to us to be important. SCZ has been consistently associated with a range of early neurodevelopmental abnormalities (Murray and Lewis, 1987; Weinberger, 1987; Seidman, 1990). One measure that may reflect early neurodevelopmental

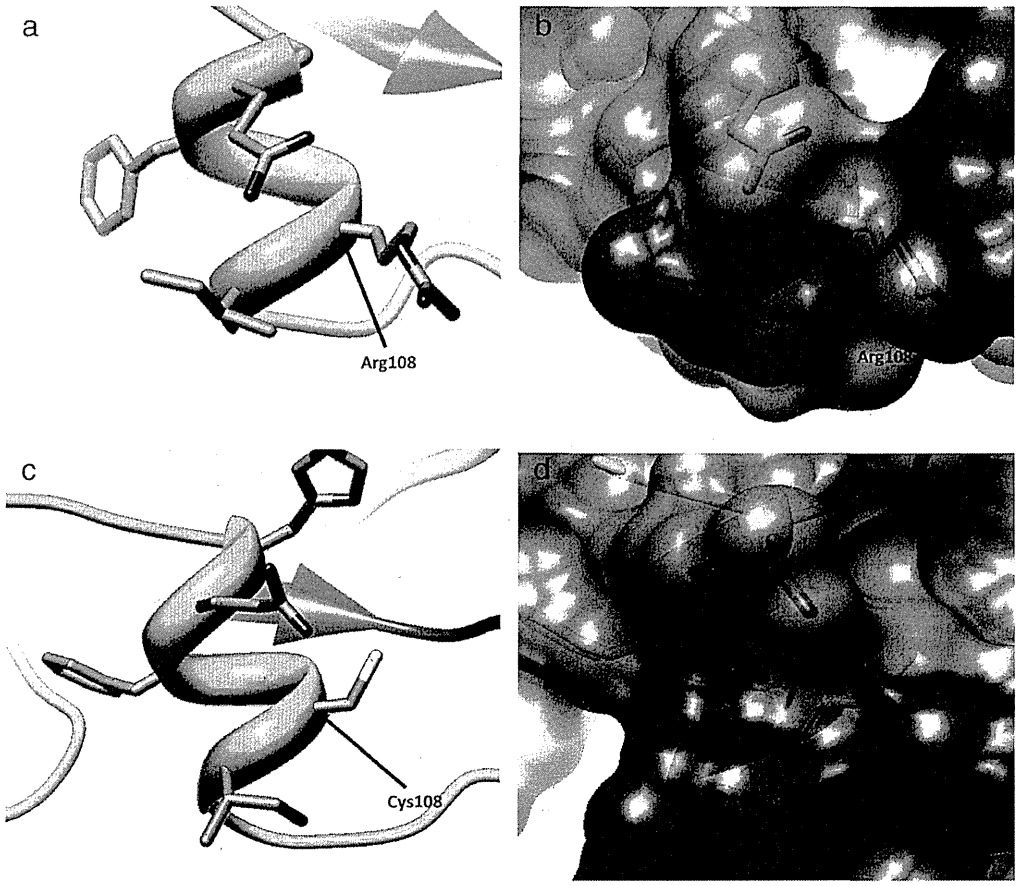

Fig. 2. 3D protein modeling of the alpha helix adjacent to the SPRY domain (FBXO45). Legend: (a) Wild type; note Arg108 on the hydrophilic chain (blue line); (b) wild type surface; note Arg108 on the hydrophilic surface (transparent blue); (c) mutant; note Cys108 on the hydrophobic chain (yellow line); (d) mutant surface; note Cys108 on the hydrophobic surface (transparent orange).

abnormalities and that has received considerable attention is premorbid intelligence (premorbid IQ) (Woodberry et al., 2008; Khandaker et al., 2011). The premorbid IQ was estimated to be 88 with JART, which was a decrease of more than 1 S.D. from the mean premorbid IQ of SCZ patients (102 ± 11.6) and controls (102.3 ± 7.4) (Hori et al., 2008). The patient's score on the Token motor test from BACS-J was

Table 2
Multiple alignments of FBXO45's homologous proteins and F-box proteins.

Homologue	Start																															End
NP_498046.1 C. elegans	146	L	G	S	A	K	K	K	L	R	A	W	Y	F	A	W	N	T	S	D	I	S	R	N	N	Y	I	R	T	N	G	F	T	V	H	179
NP_956889.1 zebrafish	105	L	A	S	Y	K	G	K	L	K	S	F	Q	H	A	L	S	S	H	D	C	S	R	N	V	Y	I	K	K	N	G	F	T	L	H	138
XP_001232841.2 chicken	118	V	P	T	Y	K	G	K	V	R	A	F	H	H	A	F	S	T	N	D	C	S	R	N	V	Y	I	K	K	N	G	F	T	L	H	151
XP_851899.2 dog	97	L	P	S	Y	K	A	K	V	R	A	F	Q	H	A	F	S	T	N	D	C	S	R	N	V	Y	I	K	K	N	G	F	T	L	H	130
NP_775615.2 mouse	100	L	P	S	Y	K	A	K	V	R	A	F	Q	H	A	F	S	T	N	D	C	S	R	N	V	Y	I	K	K	N	G	F	T	L	H	133
NP_001193687.1 cow	95	L	P	S	Y	K	A	K	I	R	A	F	Q	H	A	F	S	T	N	D	C	S	R	N	V	Y	I	K	K	N	G	F	T	L	H	128
NP_001099043.1 human	100	L	P	S	Y	K	A	K	I	R	A	F	Q	H	A	F	S	T	N	D	C	S	R	N	V	Y	I	K	K	N	G	F	T	L	H	133
XP_002803137.1 rhesus monkey	100	L	P	S	Y	K	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	104
NP_610849.1 Drosophila	69	V	S	T	Y	K	T	K	L	R	A	Y	F	H	A	W	S	P	N	D	C	S	R	N	V	Y	I	K	P	N	G	F	T	L	H	102
XP_309899.3 A. gambie	72	V	P	T	Y	K	A	K	L	R	A	Y	F	H	A	W	N	P	F	D	C	S	R	H	V	Y	I	K	P	N	G	F	T	L	H	105

F-box protein	F-box													Linker																	
FBXO45	K	H	W	Y	R	C	L	H	G	D	E	N	S	E	V	W	R	S	L	C	A	R	S	L	A	E	E	A	L	–	–	–	R	T
Fbxw7	R	Y	W	–	R	I	L	A	–	–	E	D	N	L	L	W	R	E	K	C	K	E	E	G	I	D	E	P	L	H	I	K	R	R
Skp2	K	R	W	–	Y	R	L	A	–	–	S	D	E	S	L	W	Q	T	L	–	–	–	–	D	–	–	–	–	L	T	G	K	N	

Linker																															
FBXO45	D	I	L	–	–	–	–	C	N	L	P	S	Y	K	A	K	I	R	A	F	–	Q	H	A	F	S	T	N	D	C	S	R	N	V
Fbxw7	K	V	I	K	P	G	F	I	H	S	P	W	K	S	A	Y	I	R	–	–	–	Q	H	R	I	D	T	N	W	R	R	G	E	–
Skp2		L								H	P	D	V		T		G	R	L	L	S	Q	G	V	I			A	F	R	C	P	R	S

Arginine

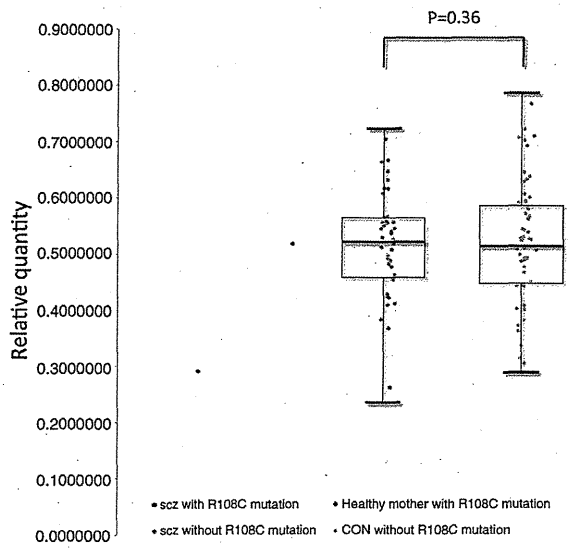

Fig. 3. Relative expression of FBXO45. Legend: Box plot: the box represents the middle 50% of observations. The middle bold line represents the median gene expression. Whiskers represent the minimum and maximum observations. Each dot represents the relative expression of each sample, which was calculated with the $2^{-\Delta\Delta C_T}$ method. The relative expression of SCZ patient with R108C mutation was an outlier of the entire sample set, while his healthy mother with the same mutation had no deviation of gene expression. The relative expression of FBXO45 was not significantly different between the 50 SCZ patients and the 52 controls ($P = 0.36$, directional test, with the Mann–Whitney U test).

less than 1 S.D. away from the mean score of SCZ patients, meaning that the patient may have a severe deficiency in motor speed (Table S1 and Fig. S2) (Kaneda et al., 2007). The CPT-IP score in this patient was less than 1 S.D. away from the mean score of SCZ patients, indicating that he may suffer from serious attention/vigilance deficits (Table S2 and Fig. S3) (Koide et al., 2012).

As a result of the pedigree study, we found that the mother of the proband was a heterozygous carrier of the R108C mutation; the DNA of the father was not available. The R108C mutation of the proband may have been inherited from the unaffected healthy mother because frequency of the mutation in cases and controls was very rare (MAF < 0.001). In other words, it is much less likely that the father was a carrier of the R108C mutation who transmitted it to the proband. If the R108C mutation was inherited from both parents, this pedigree case showed incomplete penetrance of this mutation regarding the SCZ phenotype.

The R108C mutation changed the hydrophobic distribution of the amino acids in the FBXO45 protein as predicted by structural analysis (Fig. 2). The R108C mutation was located in an evolutionarily conserved region (Table 2) between the F-box domain and the SPRY domain (Fig. 1b). The R108C mutation was predicted to be a damaging mutation for FBXO45 protein function by two kinds of algorithms (PMut and Polyphen-2). Because our sequence alignment of F-box protein family members and structural analysis of FBXO45 suggested that the mutation was located in the flexible linker region, we speculate that it may be relevant to our understanding of how the function of FBXO45 is affected by the R108C mutation (supplements). Hence, R108C is likely to play a role in a conformational change that prevents FBXO45 from correctly forming the ubiquitin ligase complex, further impacting ubiquitylation proteolysis and disrupting synaptic function.

Because genetic variation affects disease susceptibility in two ways (affecting the structure of the encoded protein and expression of the gene), thereby changing the amount or distribution of the protein (Harrison and Weinberger, 2005), we further conducted gene expression analysis of LCLs. Interestingly, the patient in whom we detected the R108C mutation had much lower *FBXO45* expression compared to the 50 SCZ patients and the 52 healthy controls, while in the case of his healthy mother who is also R108C mutation carrier, *FBXO45* expression was not reduced (Fig. 3). This observation implies that R108C mutation might not be a causal variant affecting gene expression of *FBXO45*. However, we cannot rule out the possibility that down-regulation of *FBXO45* in this patient with the R108C mutation might be relevant to the R108C mutation along with other joint factors, such as the effect of epistasis (Lappalainen et al., 2013), long-term antipsychotic medication (Hashimoto et al., 2004). Because of only two samples with R108C mutation, an estimation of R108C transcriptional influence using mRNA expression analysis seemed to be a coin-tossing situation. It would be useful for further estimation to increase genotype sample set in order to discover more R108C carriers.

In addition, we also identified one synonymous and two intronic variants. They were excluded from our follow-up analyses because our study design focused on putatively functional variants in exons. However, it may be premature to conclude that "non-functional" variants have no effect on protein function. A growing amount of evidence suggests that synonymous mutations may not always be silent; they may influence the abundance and function of proteins by activating cryptic splicing sites, affecting the stability of the mRNA, or altering the protein-folding pathway (Plotkin and Kudla, 2011; Sauna and Kimchi-Sarfaty, 2011).

4.2. Limitations

This study has several limitations. First, the sample size of our study was relatively small and lacked the statistical power to detect an association between the very rare variant R108C in *FBXO45* and SCZ. Second, several potentially valuable regions were not sequenced, including the promoter and the 5′- and 3′-UTR ends. Third, we did not validate the pathological effect of the R108C mutation with a biological experiment. Fourth, *FBXO45* gene expression profiling was evaluated using LCLs from a small sample size, and neuronal tissues were not examined. Because of controversy involving the use of non-neuronal tissues for detecting gene expression differences associated with predisposition to SCZ (Matigian et al., 2008), *FBXO45* expression should be examined in the central nervous system, and a larger sample size should be examined. In addition it would be interesting to investigate polygenic risk burden of the individuals selected for resequencing. If the case with the candidate rare variant has a low polygenic risk score, it might suggest that this variant is more likely to be causative.

5. Conclusion

The novel, heterozygous, rare, and missense mutation R108C was discovered using our mutation screening. This mutation may have a potentially pathogenic effect on FBXO45 protein structure and function, and thus, the variant may have a modest effect on SCZ risk. In addition, our findings suggest that *FBXO45* may be a new and interesting candidate gene for SCZ.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2014.04.032.

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Contributors

Conceived and designed the experiments: CW TK BA. Performed the experiments: CW HK YN AM AY. Analyzed the data: CW TK AY MB BA. Contributed reagents/materials/analysis tools: HK TK AY YN NK YT JX BA MI TO Tli Tin NI. Wrote the paper: CW TK SK IK BA NO.

Conflict of interest

The authors declare that they have no competing financial or other interests that might be perceived to influence the results and discussion reported in this paper.

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9．薬物療法

岡田 俊*

Ⅰ．はじめに

自閉スペクトラム症（ASD）に対する薬物療法は，少数例において有効性が示唆された薬剤が，その後に統制群を設定した厳密な検討において有効性が否定されるという歴史を繰り返してきた。また，薬物療法の標的を明確にせず，「問題行動」の背景を明確にしないまま鎮静を主眼とした薬物療法を行うことへの批判も強かった。これらの歴史が示唆するところは，まず標的とする問題について，その背景を明確にし，適切な環境調整や行動面からのアプローチを行うこと，薬物療法を実施するにあたり標的を明確にしてエビデンスに基づく薬物療法を実施することの重要性である。ASDの人が，定型発達者に比べて副作用を言語化しにくいこと，そのために副作用が行動面や情緒面の問題として表現されがちであることなどを考慮すると，副作用のなるべく少ない薬剤を適正な用量で使用し，その後の副作用の有無に注意を払うこと，薬剤の使用にあたっては，短期的・長期的なリスク・ベネフィットを十分に検討することが求められる。

近年の薬物療法は，その有効性の追求よりも，忍容性のより高い薬剤の開発を主眼に進められてきた。このことがASDの薬物療法の敷居をより低くしてきたことは否めない。たとえば，児童・青年に対する抗精神病薬の使用状況を調べた米国の研究では，1993～1995年に比べて2002年には6倍に増加しており，92.3%は第二世代抗精神病薬であった[23]。また，精神病性障害よりも，破壊的行動障害，抑うつ障害や双極性障害，ASDや知的能力障害，チック症などに多く使用されている。しかし，非精神病性障害への抗精神病薬の投与を正当化するエビデンスは必ずしも十分ではなく，米国精神医学会は「精神病性障害以外の診断のもと，児童・青年の第一次治療として抗精神病薬を処方すべきではない」との注意喚起を出している[1]。しかし，現実には，ASDの易刺激性や興奮性に対して，risperidone（RIS）やaripiprazole（APZ）が米国食品医薬品局の承認薬となっており，広く臨床使用されている。本稿では，二重盲検比較試験に基づいて，ASDの薬物療法のエビデンスで何が明らかにされているかを述べる。

Ⅱ．注意欠如・多動症治療薬

1．Methylphenidate

ASDに不注意，多動性-衝動性を伴うことはしばしばであり，併存例では日常生活上の困難が大きく，注意欠如・多動症（ADHD）治療薬が有効な例も多い。しかしASDとADHDの併存診断が認められたのはDSM-5以降のことである。

自閉性障害の児童・青年を対象にした4つの臨床研究がある。ADHD症状を併存する自閉性障害の7～11歳の10人の児童（平均（標準偏差）：8.5（1.3）歳；発達指数（DQ（標準偏差））：64.3（9.9）；男児6人，女児4人）について，プラセボとmethylphenidate（MPH）10/20mg（1日2回）の二重盲検クロスオーバー法（各2週間）で試験を実施した。その結果，プラセボ群に比べてMPH群の方が有意な改善を示し，常同性の増強

*名古屋大学医学部附属病院親と子どもの心療科
〔〒466-8550 愛知県名古屋市昭和区鶴舞町65〕
Takashi Okada, M.D., Ph.D.: Department of Child and Adolescent Psychiatry, Nagoya University Graduate School of Medicine, 65, Tsurumai-cho, Showa-ku, Nagoya-shi, Aichi, 466-8550 Japan.

などの副作用はみられなかった[25]。

ADHD症状を伴う自閉性障害／特定不能の広汎性発達障害のある5.6～11.2歳の児童13人（平均知能から重度／最重度知的障害までを含む：男児10人，女児3人）をプラセボとMPH 0.3/0.6mg/kgの二重盲検クロスオーバー法（各1週間）で試験を実施した。その結果，プラセボに比べてMPH投与下ではADHD症状（Conners評価尺度）と異常行動チェックリスト（ABC）のうち2項目（多動，不適切な発語）において有意な改善を認めたが，自閉症症状（CARS）には変化を認めなかった。MPHを投与中に，社会的ひきこもり，易刺激性を認めた児童がおり，特に0.6mg/kg投与時に多くみられたことから副作用の発現に注意が必要と思われた[7]。

二重盲検プラセボ対照クロスオーバー試験（4週間）とそれに続く非盲検継続試験（8週間）では，中等度から重度の多動性を有する72人の5～14歳のASDの児童（平均（標準偏差）7.5（2.2）歳，男児59人，女児13人）が参加した。プラセボ，あるいは低用量，中用量，高用量（体重によりにそれぞれの投与量は異なる）のMPHが投与され，臨床全般改善度（CGI）ならびに親，教師評価ABCスコアでアウトカムを評価した。その結果，MPHは，プラセボに勝る有効性を示し（エフェクトサイズ（ES）0.20～0.54），奏効率は49%であり，有害事象による中断が13%であり，ADHD単独例に比べて有効性が低く，有害事象による中断が多いことが示唆された[27]。また，このデータを二次解析し，評価尺度によって対人コミュニケーション（共同注意の開始，反応，要請），競合する要請課題時の自己制御・感情統制能力を調べた研究（n=33，5～13歳）では，MPHがこれらの改善に有効であることが示唆されている[15]。

ADHD症状を伴うASD（n=12）または知的障害（n=2）の14人の就学前児童（3～5歳）に対し，MPHの至適用量を決定した後，4週間の二重盲検プラセボ対照クロスオーバー試験（プラセボ2週間と至適用量のMPH 2週間）を実施した。その結果，親評価のConners尺度の得点は発達障害群でプラセボ群に比べて有意な改善を示した（p=0.005，Cohen d=0.97；発達障害全体ではp=0.08にとどまった）。しかし，半数で，常同行動の増加，胃部不快感，睡眠障害，易刺激性などの有害事象が認められ，1人は有害事象のために中止した[6]。

これらの試験のメタ解析[36]から，ASDに併存するADHD症状に対しMPHは有意な改善を示し（ES=0.6，p<0.05），その中でも多動性（ES=0.67，p<0.001）に有効である。その他，有意差には至らないも易刺激性（ES=0.52，p=0.08），常同性（ES=0.47，p=0.11）に対する効果も示唆される。副作用としては食欲低下（絶対リスク差（ARD）=0.17，害必要数（NNH）=5.9，p<0.05），不眠の増強（ARD=0.19，NNH=5.3，p<0.05），抑うつ症状の増強（ARD=0.07，NNH=14.3，p<0.05），易刺激性の増強（ARD=0.14，NNH=7.1，p<0.01），社会的ひきこもりの増強（ARD=0.07，NNH=14.3，p<0.05）が認められた。

2．Atomoxetine

ADHD症状を伴うASDの5～15歳の児童16人を対象に，atomoxetine（ATX）とプラセボを1週間のウォッシュアウト期間を挟んで各々6週間ずつ二重盲検クロスオーバー法で投与し，その有効性を比較した。その結果，ABCの多動性サブスコアにおいてATXはプラセボに比べて有意な改善を示し（p=.043，d=0.90），DSM-Ⅳの多動性-衝動性の9項目においても有意な改善がみられたが（p=.005，d=1.27），不注意項目では有意差には至らなかった（p=.053，d=0.89）。忍容性は高く，常同性の増強はみられなかった[2]。

Harfterkampらは，6～17歳のADHD症状を併存するASD児童97人をプラセボと1.2mg/kg/日のATXに無作為割り付けし，8週間の治療効果をADHD-RS，CGI-I，教師評価Conners尺度短縮版を用いて比較した。その結果，ATX群は40.7→31.6，プラセボ群は38.6→38.3へと改善し，有意差が認められた（p<.001）。また，教師評価では多動性のみATX群でプラセボ群に比べて有意な改善を認めた。有害事象の発現率はATX群の81.3%（プラセボ群は65.3%，n.s.）に認められ，多くは嘔気，食欲低下，倦怠感，早朝覚醒であった[8]。

3．α2アゴニスト

自閉症の9人（5～33歳；すべて男性）を対象に二重盲検クロスオーバー法を用いて，経皮的clonidine（約0.005mg/kg/日）とプラセボを，2週間のウォッシュアウト期間を挟んで4週間ずつ投与し，親評価と医師評価で効果を調べた。その結果，Ritvo-Freeman Real Life Rating Scaleのうちの3つの下位尺度（対人関係，情緒反応，感覚反応）ならびにCGIで，プラセボに比べて有意な改善を示した。投与から2週間の間に鎮静と倦怠感が認められた[1]。

4．まとめ

これまでのエビデンスは，多くがプラセボ対照二重盲検クロスオーバー試験であり，症例数も比較的少ない。主に多動性-衝動性に有効であるが，その有効率はADHD単独例に比べて低く，副作用も出現しやすい。

Ⅲ．第二世代抗精神病薬

1．Risperidone

自閉性障害（17人）または特定不能の広汎性発達障害（14人）の成人31人（平均（標準偏差）28.1（7.3）歳）を対象に12週間のプラセボ対照二重盲検比較試験を実施した（RIS投与量（標準偏差）2.9（1.4）mg/日，1～6mg/日）。奏効したのはRIS群の57%，プラセボ群の0%であり，攻撃性（$p<0.001$），易刺激性（$p<0.01$），反復行動（$p<0.001$），抑うつ（$p<0.03$），不安と神経質（$p<0.02$）であり，副作用として軽度の一過性の鎮静が認められた[21]。

101人の自閉症児（平均年齢（標準偏差）8.8（2.7）歳）を対象とした8週間のプラセボ対照二重盲検比較試験を実施した（RIS（標準偏差）1.8（0.7）mg/日，0.5～3.5mg/日）。その結果，易刺激性（RIS群56.9%，プラセボ群の14.1%，$p<0.001$），臨床全般改善度（RIS群の69%，プラセボ群の12%，$p<0.001$）が改善したが，体重増加（RIS群2.7kg対PBO群0.8kg），食思亢進，全身倦怠感，眠気，めまい，流涎といった副作用が認められた[19]。その後，RISが奏効した34人とプラセボに無反応で，RISを投与して奏効した29人の計63人について16週間の追跡試験を実施しRIS（2.1（0.8）mg/日）の効果を調べたところ，その効果は長期に持続していた[22]。

79人のASD児童（5～12歳）を対象にプラセボを対照としてRIS内用液（0.01～0.06mg/kg/日，平均1.17mg/日）の効果を8週間にわたり検証した。その結果，易刺激性（RIS群の64%，プラセボ群の31%が改善）を改善し，行動障害，不安，多動，感覚過敏にも改善がみられた。副作用として，体重増加（RIS 2.7kg対プラセボ1.0kg），傾眠，食思亢進が認められた[23]。

2．Aripiprazole

癇癪，攻撃性，自傷行為などを伴う自閉症の児童・青年98人（6～17歳）を対象にAPZ群（5mg，10mg，15mgの可変用量で，標的用量は33%が5mg，41%が10mg，21%が15mg）の有効性をプラセボと比較した8週間の二重盲検試験ではAPZ群はプラセボ群に比べ1週目より認められた保護者評価のABC興奮性スコアの有意な改善が8週目まで持続し（それぞれ-12.9，-5.0，d=-7.9［95%CI：-11.7- -4.1］，$p<0.001$），そのESは0.87であった。同様に，CGI（医師評価）も1週目よりAPZ群で有意な改善を示して8週目まで持続した（2.2，3.6，d=-1.4［95%CI：-1.9- -1.0］，$p<0.001$）ほか，治療反応（ABC興奮性スコアが25%改善，CGI改善度スコアが2以下）を示した患児の割合も2週目よりAPZ群が有意に多くなり，8週目ではプラセボ群の14.3%に比べAPZ群は52.2%であった（$p<0.001$）。主な有害事象は疲労，傾眠，錐体外路症状などであった。また，8週目における有害事象による治療脱落率はプラセボ群の6.0%に対してAPZ群は10.6%であり，体重増加もプラセボ群に比べてAPZ群で有意に高いという結果であった（LOCF解析でそれぞれ2.0kg，0.8kg，$p<0.005$）[24]。

癇癪，攻撃性，自傷行為などの問題行動を呈する自閉症の児童・青年218人（6～17歳）を対象としたAPZ（2mg開始，5mg，10mg，15mgが標的用量）の有効性と安全性を検討した8週間のプラセボ対照二重盲検では，ABC興奮性スコア（保護者評価）の有意な改善は，15mg群では1週目から，5mg群および10mg群は2週目より認められ，8週目まで持続した。また，全実薬群

で8週目のCGI（医師評価）がプラセボ群に比べて有意に低かった。有害事象はほとんどが軽度または中等度のもので，治療脱落率はプラセボ群の7.7%に対して5mg群が9.4%，10mg群が13.6%，15mg群が7.4%と同等またはやや高く，治療中断に至る主な事象は鎮静，流涎，振戦であった。体重増加はプラセボ群の0.3kg増に対して，実薬群では1.3～1.5kg増と有意に高かった（いずれの実薬群も$p<0.05$）[17]。

上述の2試験データを併合してABC興奮性スコア下位項目についてpost hoc解析したところ，APZの固定用量群と可変用量群のいずれもABC興奮性スコアならびにそのうち癇癪に関連する下位項目を有意に改善した。さらに，両群ともABC常同行動スコアおよびABC多動スコアに対して一貫して有効性を示した[18]。

3．Olanzapine

ASDのある11人（6～14歳）を対象に，8週間にわたるプラセボとolanzapine（OLZ）の二重盲検クロスオーバー試験を実施した。その結果，OLZ服用時にはプラセボ服用時に比べてCGIで改善がみられ，反応者の割合も50%（プラセボ20%）と高かった。しかし，OLZ服用時には7.5（4.8）ポンド（プラセボ1.5（1.5）ポンド）の体重増加が認められた[11]。

4．まとめ

抗精神病薬の投与は，易刺激性に有効であるが，1）低用量で奏効していること（RIS 1～3mg/日，APZ 5～15mg/日），2）体重増加をきたしやすいこと（そのリスクは薬剤間に相違があるが，最もリスクの低いAPZでも増加する）が明らかとなった。

Ⅳ．新規抗うつ薬

1．Fluoxetine

ASDの45人の児童に対し，fluoxetine（FLX）液剤（最終投与量（標準偏差）9.9(4.35)mg/日）とプラセボをそれぞれ8週間ずつ投与する二重盲検プラセボ対照クロスオーバー試験を実施した。その結果，FLX服用時に小児Yale-Brown強迫症状尺度（CY-BOCS）の強迫行動スコアがプラセボ群に比べて有意な改善を示したが，全般的な改善ではわずかな差にとどまった。副作用発現率に差はなかった[10]。

ASDの成人37人を，FLX群（n=22，10～80mg/日）とプラセボ群（n=15）に無作為に割り付け，12週間の治療効果を比較した。Y-BOCSを用いて反復的行動を調べた。Y-BOCSあるいはCGIでみた反応者の割合は，それぞれFLX群が35%（PBO群は0%），FLX群が50%（プラセボ群は8%）と有意な改善を示した[14]。

2．Fluvoxamine

自閉性障害のある30人の成人をプラセボ群とfluvoxamine群に割り付け，12週間の治療効果を比較した。反応率は，fluvoxamine群は53%（プラセボ群0%）で有意に高く（$p=0.001$），反復的考えと行動（$p<0.001$），不適応行動（$p<0.001$），攻撃性（$p<0.03$），対人関係（$p<0.04$），特に言語使用（$p<0.008$）に有意な改善がみられた。副作用として鎮静と嘔気が認められた[20]。

3．Citalopram

6機関共同でASDの診断を受けCGIが中等度以上の149人の5～17歳の児童（9.4（3.1）歳）を無作為に割り付け，citalopram群（n=73，平均16.5（6.5）mg）とプラセボ群（n=76）に分け12週間の治療効果をCY-BOCSで評価した。その結果，CGI，CY-BOCSともに両群間に有意差を認めなかった。Citalopram群は，エネルギー水準の増大，衝動性の増加，集中力減退，多動，常同性，下痢，不眠，皮膚乾燥，掻痒感と関連していた[16]。

4．まとめ

選択的セロトニン再取り込み阻害薬の投与が強迫症状に有効であったという報告と，わずかに有効，あるいは有効ではないという報告があり一貫しない。エネルギー水準の増大，衝動性の増加などが認められるとの報告があり，リスク・ベネフィット，投与後の変化に十分留意する必要がある。

Ⅴ．抗てんかん薬

1．バルプロ酸

広汎性発達障害＋顕著な攻撃性のある6～20歳の30人（男児20人，女児10人）を無作為にバルプロ酸（4週時点で75.5mcg/ml，8週時点で

77.8mcg/ml）とプラセボ群に割り付け，8週間の治療を行った。その結果，ABCの易刺激性サブスケール，CGIともに有意差を認めなかった。食欲の増進，紅斑，血中アンモニアの上昇，発語不明瞭，軽度認知機能低下が認められた[9]。

ASDのある5～17歳の児童と40歳の成人1人の合計13人を対象に，プラセボ群とdivalproex群に無作為に割り付け，8週間の治療効果を調べた。その結果，CY-BOCSで評価した反復的行動がdivalproex群ではプラセボ群に比べて有意に改善した（p=0.037，d=1.616）[12]。

ASDの27人の児童（平均（標準偏差）9.46（2.46）歳；OQ（標準偏差）63.3(23.9)）を無作為に2群に割り付け，divalproexまたはプラセボを12週間にわたり投与した。その結果，反応者はdivalproex群の62.5%（プラセボ群9%）であり，CGI-易刺激性（オッズ比：16.7，Fisher's exact p=0.008），ABC-易刺激性スコア（p=0.048）にも有意な改善が認められた[13]。

2．まとめ

バルプロ酸の投与が反復的行動または易刺激性に有効であったという報告と，有効性を示せなかった報告があり一貫しない。

Ⅵ．睡眠薬

1．メラトニン

11人のASD児童を対象に，無作為割り付け二重盲検クロスオーバー法でプラセボ，メラトニンを投与したところ，プラセボに比べてメラトニン投与時には，睡眠潜時が短縮，中途覚醒回数が減少，総睡眠時間が延長した[5]。

ASDの児童・青年51人（2～18歳）を対象に，3ヵ月の非盲検試験の後，徐放性メラトニン5mgまたはプラセボを投与し，親記入の睡眠記録とアクティグラフで睡眠状態を評価した。その結果，総睡眠時間を延長し，睡眠潜時を約30分短縮した[29]。

22人のASD児童に，二重盲検クロスオーバー法を用いて，プラセボ，メラトニン（最大10mg）を投与したところ，総睡眠時間が平均52分延長し，睡眠潜時が平均47分短縮した[30]。

4～10歳のASD児童160人を（1）徐放性メラトニンと認知行動療法，（2）徐放性メラトニン，（3）認知行動療法，（4）プラセボに無作為割り付けし，12週間の治療を行い，アクティグラフ，睡眠日誌，睡眠質問紙によって評価した。その結果，徐放性メラトニンは睡眠時間を延長し，認知行動療法は睡眠潜時を短縮した。さらに，併用療法が最も良好な効果を示し，標準睡眠効果基準＞85%が63.38%，30分未満の睡眠潜時が84.62%に認められた[3]。

2．まとめ

メラトニンの投与が，ASDに併存する睡眠障害に対し，睡眠時間の延長，睡眠潜時の短縮をもたらすことが示されている。

Ⅶ．おわりに

以上，これまでに提出されているASDの薬物療法のエビデンスを概観したが，これらはそのすべてがASDの関連症状あるいは併存障害に対する薬物療法であり，中核障害の治療薬は確立していないことに留意する必要がある。

ADHD治療薬は，ASDに併存する不注意・多動性-衝動性を改善し，抗精神病薬の投与はASDの易刺激性や興奮性を改善するが，副作用の発現率も高く，短期的・長期的なリスク・ベネフィットが考慮されなければならない。新規抗うつ薬や抗てんかん薬の有効性は一貫して示されていない。睡眠薬については，メラトニンの有効性は示されるも，その他の睡眠薬のエビデンスは皆無である。

いずれにせよ，中核症状を治療する薬剤がない以上，薬物療法は補助治療である。環境調整，行動的アプローチ，心理的支持などとともに多面的な治療の一助として位置づけられることを心得る必要がある。

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特集

ライフサイクルに応じた統合失調症の薬物療法

児童青年期の統合失調症の薬物療法

岡田　俊*

抄録：統合失調症を顕在発症した児童・青年を対象にした二重盲検比較試験を展望した。児童青年期の統合失調症には，非定型抗精神病薬の定型抗精神病薬に対する優位性が認められず，また非定型抗精神病薬間でも比較試験において有意な差を認めていない。しかし，錐体外路性副作用の出現は定型抗精神病薬に多く，代謝系副作用は olanzapine，quetiapine，risperidone，clozapine で多く，aripiprazole で少ない。また，aripiprazole はプロラクチンを上昇させない，など，薬剤間で副作用のプロフィールには相違がある。臨床効果よりも副作用に基づく薬剤選択が現実的である。しかし，これまでのエビデンスは，ほぼ青年期のものであり，児童期の薬物療法がいかにあるべきか検討が求められる。

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Key words : *early-onset schizophrenia, childhood and adolescent, neuroleptics, efficacy, adverse event*

Ⅰ．はじめに

統合失調症の好発年齢は20～30歳代とされるが，成人期の発症例においても児童青年期から認知機能変化や非特異的な行動変化が認められ，今日では統合失調症は児童青年期から出現する神経生物学的プロセスとの見方がなされている。同時に，児童青年期に顕在発症する早期発症統合失調症も決して稀ではなく，13歳以前に発症する超早期発症統合失調症の有病率は0.0019％に留まるが，青年期発症統合失調症は0.23％に及ぶ[8]。

児童青年期の統合失調症は，幻視が多い，空想的，魔術的な妄想が多く，その構造は浮動的で体系化されることが少ない，はっきりとした幻覚や妄想が認められないこともある，人格水準の低下が進行する場合，生活能力の低下が重篤になりやすい，といった特徴があり，統合失調症の中で診断が困難で，かつ，重症な一群として認識されてきた。治療に関するエビデンスは急速に増えつつあるものの，いまなお成人に比べて不十分であり，その治療は成人のエビデンスに倣って実施されてきたのが現状である。

本稿では，統合失調症を顕在発症した児童・青年の無作為割り付け比較対照試験のエビデンスに限定して検討を加え，児童青年期の統合失調症治療のリスク・ベネフィットとその課題について明らかにする。

Ⅱ．プラセボとの無作為割付比較対照試験

1．Risperidone

統合失調症急性増悪期の青年（13～17歳）160人をプラセボ群（n＝54），risperidone 1～3mg 群

Pharmacotherapy for schizophrenia in childhood and adolescence.

*名古屋大学医学部附属病院親と子どもの心療科
〔〒466-8550　愛知県名古屋市昭和区鶴舞町65〕
Takashi Okada：Department of Child and Adolescent Psychiatry, Nagoya University Graduate School of Medicine. 65 Tsurumaicho, Showa-ku, Nagoya, Aichi, 466-8550, Japan.

(n＝55)，risperidone 4～6mg群（n＝51）に割り付け，PANSSスコアと反応率（PANSSスコアの20％以上の低下）により6週間の有効性を評価した。その結果，プラセボ群（－8.9±16.1）に比べてrisperidone 1～3mg群（－21.3±19.6），risperidone 4～6mg群（－21.2±18.3）は有意な改善を示し（p<0.001），改善率もそれぞれ35％，65％，72％であった。有害事象は，プラセボ群（54％）よりもrisperidone 1～3mg群（75％），risperidone 4～6mg群（76％）で多く，特にrisperidone 4～6mg群では1～3mgよりも錐体外路症状，めまい，倦怠感などが多くみられた[6]。

統合失調症の急性エピソードの青年（13～17歳）を，risperidone 1.5～6mg群（n＝125）とrisperidone 0.15～0.6mg群（n＝132）に無作為割り付けし，8週間の効果を比較した。その結果，両群ともベースラインに比べてPANSSスコアの有意な改善を示した（P<0.001；effect size＝0.49；1.5～6mg 96.4±15.39→72.8±22.52，0.15～0.6mg 93.3±14.14→80.8±24.33）。1.5～6mg群の方がベースラインからの改善は大きかったが，0.15～0.6mg群との間に有意差は認めなかった。有害事象は，1.5～6mg群の74％，0.15～0.6mg群の65％に認められ，体重は1.5～6mg群が3.2±3.49kg，0.15～0.6mg群が1.7±3.29kgの増加であった[7]。

2．Paliperidone

統合失調症の青年（12～17歳）201人を，プラセボ群，paliperidone低用量群，中用量群，高用量群（体重が29～51kgの場合には低用量1.5mg，中用量3mg，高用量6mg，体重が51kgを越える場合には，低用量1.5mg，中用量6mg，高用量12mg）に人数比が1：1：1：1になるように無作為割り付けし，PANSSスコアのベースラインからの改善を指標として6週間の治療効果を比較した。その結果，プラセボ群（－7.9±20.15；n＝51）に比べて，中用量群（－17.3±14.33；p<0.05；n＝54）は有意な改善を示したが，低用量群（－9.8±16.31；n＝48）と高用量群（－13.8±15.74；n＝47）は有意な改善を示さなかった。用量別では，プラセボ群（－7.9±20.15）に比べて3mg（－19.0±15.45），6mg（－13.8±14.75），12mg（－16.3±15.41）とも有意な改善を示した（all ps<0.05）。忍容性は高かった[19]。

3．Olanzapine

統合失調症の青年107人をolanzapine群（n＝72，平均16.1歳）とプラセボ群（n＝35，平均16.3歳）に人数比が2：1になるように無作為割り付けし，2.5～25mg/日のflexible doseで治療を行い，6週間の効果を比較した。その結果，治験期間終了時まで服用した患者の割合が，プラセボ群に比べてolanzapine群の方が高く（68.1％対42.9％；p＝0.02），olanzapine群はプラセボ群に比べBPRS（olanzapine群－19.4，プラセボ群－9.3；p＝0.003），CGI（p＝0.04），PANSS（p＝0.005）の有意な改善がみられた。Olanzapine群ではプラセボ群に比べて有意な体重増加が認められ（4.3kg vs. 0.1kg；p<0.001），7％以上の体重増加が認められた患者の割合も有意に高かった（45.8％ vs. 14.7％；p＝0.002）。また，プラセボ群に比べてトリグリセリドとプロラクチン値が有意に高かった[9]。

4．Quetiapine

統合失調症の青年（13～17歳）220人をプラセボ群とquetiapine 400mg，800mg群に無作為割り付けし，PANSSスコアを指標として6週間の治療効果を検討した。プラセボ群（－19.15）に比べてquetiapine 400mg群（－27.31），800mg群（－28.44）は有意な改善を示し（それぞれp＝0.043，0.009），CGIでも有意な改善を認めた。体重は，プラセボ群が－0.4kgに対し，quetiapine 400mg群は＋2.2kg，800mg群は＋1.8kgであった。生化学検査では，総コレステロール値とトリグリセリドがプラセボ群に比べてquetiapine群では有意に高かった[2]。

5．Aripiprazole

統合失調症の青年302人（13～17歳，PANSSスコア70点以上，平均94.1）をプラセボ群とaripiprazole 10mg群，aripiprazole 30mg群に人数比が1：1：1になるように無作為割り付けし，6週間の効果を比較した。その結果，aripiprazole 10mg群，30mg群ともにプラセボ群に比べてPANSS ス

コアが有意に改善した（プラセボ群－21.2±1.9, aripiprazole 10mg 群 －26.7 ±1.9, 30mg 群 －28.6 ±0.9)。プラセボ群, aripiprazole 10mg 群, 30mg 群では, プロラクチン値がそれぞれ－8.45, －11.93, －15.14ng/ml, 体重変化量はそれぞれ－0.8, 0.0, 0.2kg であった[3]。

この試験を PANSS 敵意クラスタ（興奮, 敵意, 非協調性, 衝動性制御の欠如）に関してサブ解析した Robb らの研究では, 10mg 群, 30mg 群のいずれもプラセボ群に比べ PANSS 敵意クラスタを有意に改善することが報告されている（それぞれ－3.0, －3.7, 対－2.1；p<0.05)。特に, 30mg 群では3週目より PANSS 敵意クラスタの有意な改善効果が認められたほか, エンドポイントにおいて PANSS の敵意, 非協調性, 衝動性の調節障害の各項目も有意に改善されていた[14]。

6. Ziprasidone

統合失調症の青年（13～17歳）283人を人数比が2：1になるように ziprasidone 群（flexible dose, 40～160mg；n＝193）とプラセボ群（n＝90）に無作為割り付けし, 6週間の治療効果を調べ, その後26週間の非盲検延長試験を行った。その結果, 6週後の BPRS スコアの変化量は有意差がなかった（ziprasidone 群－14.16±0.78, プラセボ群－12.35±1.05；p＝0.15)。非盲検延長試験には221人が参加し, 二重盲検試験終了時より－6.9 ±8.9 の改善をみた。副作用は眠気と錐体外路症状であり, 1名が自殺した。QT 延長は認められなかった[4]。

Ⅲ. 定型抗精神病薬と非定型抗精神病薬の無作為割付比較対照試験

1. Haloperidol・risperidone・olanzapine

統合失調症の青年50人（8～19歳）を haloperidol 群, risperidone 群, olanzapine 群に無作為割り付けし, 8週間の治療効果を比較した。いずれもベースラインから BPRS スコアを有意に改善した（haloperidol 群 P＝0.012, risperidone と olanzapine 群 P＝0.012)。BPRS スコアのベースラインからの改善は, haloperidol 群 49.0±14.0→33.0±19, risperidone 群 54.0±1.3→27.0±20, olanzapine 群 50.0±10→22.0±12 で各群間に有意差を認めなかった。反応率は haloperidol 群が53％, risperidone 群が74％, olanzapine 群が88％であったが統計学的に有意な水準ではなかった。Risperidone や olanzapine を投与された患者で体重増加や錐体外路症状が認められたが, 発現頻度は成人のデータから推測されるよりも高頻度であった[17]。

2. Molindone・risperidone・olanzapine

統合失調症, あるいは統合失調感情障害と診断された青年119人（8～19歳）を olanzapine 群（2.5～20mg), risperidone 群（0.5～6mg), molindone 群（10～140mg と 1mg の benztropine）に無作為割り付けし, 8週間の治療効果を比較し, CGI-I が1または2, および PANSS の20％以上の改善によって比較した。その結果, PANSS のベースラインからの改善は, olanzapine 群が－26.6 ±17.8, risperidone 群が－23.7±25.5, molindone 群が－27.0±17.7であり, 有意差を認めなかった。改善率は olanza-pine 群34％, risperidone 群46％, molindone 群50％であり, 改善率にも有意差を認めなかった。Olanzapine 群と risperidone 群, 特に olanzapine 群では有意な体重増加と脂質異常症が認められ, molindone ではアカシジアが認められた[18]。

Ⅳ. Clozapine との無作為割付比較対照試験

1. Olanzapine

治療抵抗性（少なくとも2つの抗精神病薬に対して反応不良）の統合失調症の青年（7～16歳）を1～3週間のウォッシュアウト期間の後, olanzapine 群（n＝13）と clozapine 群（n＝12）に無作為割り付けし, 8週間の比較試験, その後, 2年間の非盲検フォローアップを実施した。その結果, すべての評価尺度で olanzapine 群より clozapine 群の方が改善したが, 有意差が認められたのは陰性症状（SANS）の改善のみであった（clozapine vs. olanzapine＝－25 vs. －14；P＝0.04；effect size 0.89)。2年間のフォローアップ期間に6人に脂質異常症, 1人にけいれんが認められた[16]。

Clozapineと高用量のolanzapineを比較した研究もある。治療抵抗性（2つ以上の抗精神病薬に反応不良）の統合失調症の青年（10～18歳）をclozapine群（n＝18）と高用量（最大30mg）のolanzapine群（n＝21）に無作為割り付けし，flexible doseで12週間の治療を行い，BPRSの30％以上で，かつ，CGI-Iで1または2で定義された改善率で評価した。その結果，改善率はclozapine群66％，olanzapine群33％であり，clozapine群の方が陰性症状（SANS）の改善において優れていた（clozapine群10.3 ±3.6 →6.6 ±4.4，olanzapine群9.4±2.7→7.6±3.8）。しかし，両群とも有意な体重増加と代謝系副作用が認められた[11]。

V．ま と め

これまでに提出された無作為割り付け二重盲検試験の結果は以下のようにまとめられる。①プラセボに比べて，定型抗精神病薬，ならびにziprasidoneを除く非定型抗精神病薬が有意な有効性を示す，②一定の用量－効果関係は認められるが，少量投与でも有効であったり，中用量の方が高い有効性が示されることもある，③定型抗精神病薬に比べ，非定型抗精神病薬が治療効果に優れることを示すエビデンスは得られていない，④非定型抗精神病薬の投与では，定型抗精神病薬に比べて，錐体外路性副作用のリスクが低いが，有意な体重増加や脂質異常症の発現など代謝系副作用が多い，プロラクチン上昇のリスクはaripiprazoleで低い，⑤非定型抗精神病薬の間で有効性の差があることは認められていない，⑥治療抵抗性統合失調症にはclozapineが優れる，ということである。

これまでの34本の臨床試験をもとにメタ解析を行い，児童青年期における体重増加，代謝系副作用のリスクを調べた研究では，olanzapineで3.8～16.2kg，clozapineで0.9～9.5kg，risperidoneで1.9～7.2kg，quetiapineで2.3～6.1kg，aripiprazoleで0～4.4kgの増加であった。7％以上の体重増加を認めるnumbers-needed-to-harm（NNH）は，aripiprazoleが39，ziprasidoneが36，quetiapineが9，risperidoneが6，olanzapineが3であった[12]。

13～18歳の青年期精神病患者における非定型抗精神病薬の有効性と安全性について検討した13の無作為化比較試験のコクランレビューによれば，clozapine，olanzapine，risperidoneは体重増加のリスクがあるが，aripiprazoleは体重増加や脂質異常のリスクが低く，プロラクチン値は上昇しない[10]という。

体重増加や脂質異常は，さまざまな内分泌代謝系疾患や冠動脈疾患のリスクファクターであり，平均余命を短縮する可能性がある。よって，代謝系副作用による長期的な健康リスクを総合的に考えた場合，非定型抗精神病薬の投与が定型抗精神病薬に比べて，リスク・ベネフィットのバランスに優れるとは言い切れない。ごく少量で錐体外路症状が出現しないと思われる用量範囲であれば定型抗精神病薬の投与が賢明であるという考えも合理的と言える。非定型抗精神病薬を投与する場合には，可能な限り肥満や代謝系副作用のリスクが低く，プロラクチンを上昇させない薬剤の投与が理想的である。実際，米国食品医薬品局や欧州医薬品局は，有効性と忍容性の高さから児童青年期における非定型抗精神病薬の使用を推奨しているが，同時に，児童・青年に非定型抗精神病薬の投与を行う場合の代謝系リスクを考慮することを強調している[5]。

非定型抗精神病薬同士の比較は，まだ少数しか行われていないが，その有効性に差は認められていない。しかし，錐体外路系副作用や代謝系副作用のリスクは非定型抗精神病薬間で明らかな相違があり，児童青年期の統合失調症治療においては，有効性以上に副作用に基づく薬剤選択が重要になると思われる。

実際，米国児童青年精神医学会が発表したPractice Parametersでは，児童青年期の統合失調症および関連障害に対して，抗精神病治療がファーストラインであり，なかでも非定型抗精神病薬が第一選択になるとしている。薬剤間での比較データが不十分なため，基本的には食品医薬品局で承認されている薬剤で，かつ安全性プロファイルを考慮して選択すべきである。特に，児童青年期患者は副作用，とりわけ代謝系の副作用に対して脆弱であるため，体重増加リスクを伴う薬剤

は第一選択薬としての使用は制限されるべきである，と述べている[13]。

さらに，非薬物療法では，認知矯正療法によってBPRSの改善はなかったものの[20]，視覚情報処理[21]，認知の柔軟性[23]，実行機能の改善[22]が非盲検試験で示されている。認知行動療法の有効性については治療群の方が通常治療のみの群に比べて生活機能や生活の質が良い傾向はあったが，有意差は認められていない[1]。今後は，これらの非薬物療法との併用療法の有効性[15]や治療上の位置づけについても検討が求められる。

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