		CT (240)	365 (0.76)	115 (0.24)		136 (56.67)	93 (38.75)	11 (4.58)	
SNP05	rs10761627		T	C		T/T	T/C	C/C	
	Intron 2	BD (237)	368 (0.78)	106 (0.22)	0.591	144 (0.61)	80 (0.34)	13 (0.05)	0.540
		CT (239)	364 (0.76)	114 (0.24)		136 (0.57)	92 (0.38)	11 (0.05)	
SNP06	rs7075904		C	G		C/C	C/G	G/G	
	Intron 2	BD (237)	405 (0.85)	69 (0.15)	0.927	173 (0.73)	59 (0.25)	5 (0.02)	0.676
		CT (240)	409 (0.85)	71 (0.15)		172 (0.72)	65 (0.27)	3 (0.01)	
SNP07	rs7070152		A	G		A/A	A/G	G/G	
	Intron 3	BD (237)	385 (0.81)	89 (0.19)	0.333	159 (0.67)	67 (0.28)	11 (0.05)	0.156
		CT (240)	377 (0.78)	103 (0.22)		145 (0.61)	87 (0.36)	8 (0.03)	
SNP08	rs3758490		T	G		T/T	T/G	G/G	
	Exon 5 (Ser/Ala)	BD (236)	323 (0.68)	149 (0.32)	0.445	115 (0.49)	93 (0.39)	28 (0.12)	0.242
		CT (240)	317 (0.66)	163 (0.34)		102 (0.43)	113 (0.47)	25 (0.10)	
SNP09	rs4746698		A	G		A/A	A/G	G/G	
	3′	BD (235)	323 (0.69)	147 (0.31)	0.333	118 (0.50)	87 (0.37)	30 (0.13)	0.086
		CT (240)	315 (0.66)	165 (0.34)		101 (0.42)	113 (0.47)	26 (0.11)	
SNP10	rs2893900		C	T		C/C	C/T	T/T	
	3′	BD (238)	438 (0.92)	38 (0.08)	0.364	202 (0.85)	34 (0.14)	2 (0.01)	0.633
		CT (240)	433 (0.90)	47 (0.10)		196 (0.82)	41 (0.17)	3 (0.01)	

BD, bipolar disorder; CT, control; the number of genotyped individuals are given in parentheses.

[a] **Number followed by frequency in parentheses.**

[b] **Fisher's Exact test.**

DBZ Gene Expression Analysis

The variability in the distribution of demographic variables across the control, schizophrenia and bipolar disorder groups are summarized in Table I. There was no significant difference ($F = 0.34$; df = 2.93; $P = 0.713$) in *DBZ* expression across the three groups (Fig. S2). No significant correlations were observed between *DBZ* expression and any of the clinical features of schizophrenia or bipolar groups.

DISCUSSION

In this study, we observed SNP- and haplotype- associations of the DISC1-interacting molecule *PCNT2* with schizophrenia. SNP04, SNP14, and SNP19 showed significant allelic and genotypic associations. The allelic association of SNP04 and the haplotypic associations involving SNP04 withstand multiple testing correction. Deviation from HWE was observed for SNP04 in schizophrenia patients; heterozygotes of patients were lower than the expected values based on HWE. Since SNP04 showed association, which withstands correction for multiple testing, the deviation from HWE in patients, but not in controls, may be viewed as an additional evidence of association. We observed a strong LD between SNPs 14 and 19. SNP19, located in exon 45, is a non-synonymous SNP with Ser/Arg substitution. Since Ser is a neutral amino acid and Arg is a strong basic amino acid, the substitution may be suggested to exert an influence on the protein structure of PCNT2. To the best of our knowledge, this is the first report of a genetic association study of *PCNT2* with schizophrenia.

Abnormalities of brain morphogenesis, especially cortical dysplasia, have been observed in individuals with partial deletion of chromosome 21q22.3 harboring the *PCNT2* gene [Yao et al., [2006]]. PCNT2, an integral component of the pericentriolar material, interacts with pericentriolar material 1 (PCM1), another member of the DISC1 interactome [Kamiya et al., [2008]], for its centrosomal localization, and to accomplish its function in microtubule organization [Balczon et al., [1994]; Doxsey et al., [1994]; Kubo et al., [1999]; Dammermann and Merdes, [2002]]. Recently, *PCM1* has been implicated in susceptibility to schizophrenia [Gurling et al., [2006]]. In the developing cerebral cortex, suppression of PCM1 has been reported to lead to neuronal migration defects [Kamiya et al., [2008]]. Thus, centrosomal proteins such as PCNT2 may be suggested to have an important role in cortical development, and therefore, in the pathogenesis of neurodevelopmental disorders like schizophrenia. The PCNT2-binding region of DISC1 overlaps with the region interacting with FEZ1 [Miyoshi et al., [2004]], a schizophrenia susceptibility gene that plays a vital role in axonal outgrowth and fasciculation [Yamada et al., [2004]].

In our previous study, elevated expression of *PCNT2* was observed in the brain and in the PBL of bipolar disorder patients, compared to controls; however, there was no significant difference in *PCNT2* expression between control and schizophrenia groups [Anitha et al., [2008]]. We suggested that a possible apoptotic process in the brain, resulting from over-expression of *PCNT2*, may underlie the pathogenesis of bipolar disorder. However, in the subsequent association study, none of the SNPs showed association with bipolar disorder.

Considering the results from the genetic analyses of *PCNT2* in bipolar disorder and schizophrenia, it might be suggested that DISC1 and its interacting molecules are involved in psychiatric symptoms that cross diagnostic boundaries. The translocation in the original Scottish family [St Clair et al., [1990]] demonstrated that the disruption of DISC1 gene, although sufficient to predispose an individual to psychiatric disorder, was, in itself, insufficient to predict any particular disorder. Although the family members showed predominantly schizophrenic symptoms, they also manifested a wide spectrum of other psychiatric phenotypes. In addition to schizophrenia, DISC1 has also been found to be associated with other neuropsychiatric disorders including bipolar disorder [Hodgkinson et al., [2004]; Thomson et al., [2005]], major depression [Hashimoto et al., [2006]], and autism [Kilpinen et al., [2008]]. Accumulating evidence show that malfunction of DISC1 lead to brain structural and functional abnormalities [Faulkner et al., [2008]; Kvajo et al., [2008]; Pletnikov et al., [2008]; Prata et al., [2008]; Szeszko et al., [2008]]. Thus, it may be suggested that the basis of the shared risk for major neurodevelopmental disorders could be centered on DISC1, while its interaction with other members of the interactome lead to different phenotypic outcomes.

Several other DISC1-interacting molecules, including phosphodiesterase 4B [PDE4B; Numata et al., [2008]], 14-3-3ε [YWHAE; Ikeda et al., [2008]], nudE nuclear distribution gene E homolog 1 [NDE1; Hennah et al., [2007]], nudE nuclear distribution gene E homolog like 1 [NDEL1; Burdick et al., [2008]], and activating transcription factor 4 [*ATF4*; Qu et al., [2008]], have been suggested as potential susceptibility genes for schizophrenia. The DISC1 interactome is expanding with the discovery of novel interacting proteins, and this information could be useful in the identification of potential pathways involving DISC1, in the pathology of major psychoses.

In this study, no significant genetic associations were observed for *DBZ*, the other DISC1-interacting molecule, with schizophrenia or with bipolar disorder; in addition, there was no significant difference between the *DBZ* mRNA levels of control, schizophrenia and bipolar postmortem brains.

In conclusion, we suggest a possible role of PCNT2 in the pathogenesis of schizophrenia. Abnormalities of PCNT2 may lead to defects in microtubule function; during the development of the central nervous system, this dysfunction might result in alterations in neuronal migration, axonal extension, and neurite outgrowth, subsequently leading to impaired neurodevelopment. Our study is limited by small sample size; therefore, the results should be treated with caution, until replication using a larger sample size. It would also be interesting to study the role of PCNT2 in neurodevelopment.

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DISC1–kendrin interaction is involved in centrosomal microtubule network formation

Shoko Shimizu [a,1], Shinsuke Matsuzaki [a,b,*,1], Tsuyoshi Hattori [a,b], Natsuko Kumamoto [a], Ko Miyoshi [c], Taiichi Katayama [d], Masaya Tohyama [a,b]

[a] Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] The Osaka-Hamamatsu Joint Research Center For Child Mental Development, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[c] Department of Brain Science, Graduate School of Medicine and Dentistry, Okayama University, 2-5-1 Shikada-chou, Okayama, Okayama 700-8558, Japan
[d] Department of Anatomy and Neuroscience, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, Shizuoka 431-3192, Japan

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ABSTRACT

Disrupted-In-Schizophrenia 1 (DISC1) was identified as a novel gene disrupted by a (1;11)(q42.1;q14.3) translocation segregating with schizophrenia, bipolar disorder and other major mental illnesses in a Scottish family. We previously identified 446–533 amino acids of DISC1 as the kendrin-binding region by means of a directed yeast two-hybrid interaction assay and showed that the DISC1–kendrin interaction is indispensable for the centrosomal localization of DISC1. In this study, to confirm the DISC1–kendrin interaction, we examined the interaction between deletion mutants of DISC1 and kendrin. Then, we demonstrated that the carboxy-terminus of DISC1 is indispensable for the interaction with kendrin. Furthermore, the immunocytochemistry revealed that the carboxy-terminus of DISC1 is also required for the centrosomal targeting of DISC1. Overexpression of the DISC1-binding region of kendrin or the DISC1 deletion mutant lacking the kendrin-binding region impairs the microtubule organization. These findings suggest that the DISC1–kendrin interaction plays a key role in the microtubule dynamics.

Schizophrenia is a severe mental disorder affecting about 1% of the population worldwide. The pathogenic mechanism of schizophrenia is thought to involve the combined effects of multiple genetic components. And although genetic linkage and association studies have identified potential susceptibility genes, the pathogenic molecular mechanism of schizophrenia has yet to be elucidated.

In a large Scottish family, a balanced (1;11)(q42.1;q14.3) translocation segregating with schizophrenia and affective disorders was identified [1,2]. The *Disrupted-In-Schizophrenia 1 (DISC1)* gene on chromosome 1 was originally identified as the gene disrupted by this translocation [2,3]. The open reading frame in DISC1 encodes a protein of 854 amino acids that has no significant homology to other known proteins. The amino-terminal region (amino acids 1–347) consists of one or more globular domains, while the helical carboxy-terminal region (amino acids 348–854) contains the translocation breakpoint and three stretches with coiled-coils that have the potential to interact with other proteins [2]. In a previous study, we identified two DISC1-binding partners, the fasciculation and elongation protein zeta-1 (FEZ1) and DISC1-binding zinc-finger protein (DBZ). Both these proteins are involved in neurite outgrowth [4,5]. In addition, several groups have identified DISC1 interaction partners such as NudE-like (NUDEL) [6–8] and lissenchphaly-1 (LIS1) [9]. NUDEL and LIS1 regulate the motor protein dynein, and the NUDEL/LIS1/dynein complex plays a key role in neuronal migration [10–13]. DISC1 has been reported to maintain the NUDEL/LIS1/dynein complex at the centrosome and to regulate neural migration [14]. On the other hand, DISC1 is also involved in the transport of either NUDEL/LIS1/14-3-3ε complex or growth factor receptor bound protein 2 (Grb2) to the axonal tips through its interaction with kinesin-1 [15,16]. Therefore, DISC1 plays crucial roles in brain development—including roles in neuronal migration, neurite outgrowth and neural maturation—through its interaction with several cytoskeletal proteins.

We previously identified the large coiled-coil centrosomal protein kendrin (the human orthologue of mouse pericentrin B) as one of the DISC1-binding partners by yeast two-hybrid screening. In the same study, we showed that DISC1 localizes to the centrosome by binding to kendrin and that the residues 446–533 of DISC1 are critical for the interaction with kendrin [17]. Kendrin mediates the nucleation of microtubules by anchoring the gamma-tubulin ring complex, which initiates the assembly of the mitotic spindle apparatus [18–21]. Furthermore, our recent study revealed that *kendrin* is associated with bipolar affective disorder [22]. And mutations in

* Corresponding author. Fax: +81 6 6879 3229.
E-mail address: s-matsuzaki@anat2.med.osaka-u.ac.jp (S. Matsuzaki).
[1] These authors contributed equally to this work.

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kendrin cause Seckel syndrome, which is characterized by microcephaly [23]. Thus, the physiological role of the DISC1–kendrin interaction at the centrosome should be involved in brain development. At present, however, little is know about this role of the DISC1–kendrin interaction.

In this paper, we present the key sequence of DISC1 responsible for the interaction with kendrin and the centrosomal targeting, and provide evidence that the DISC1–kendrin interaction plays a role in the microtubule network formation.

Materials and methods

Plasmids. Plasmids expressing full-length human *DISC1* cDNA were described previously [5]. Full-length human *DISC1* was tagged with a FLAG or HA sequence at the 3′ end and cloned into pcDNA3.1(+) (Invitrogen, Carlsbad, CA). The deletion mutants of DISC1 were constructed: DISC1 ΔKBR (amino acids 446–533 were deleted), GDBP (amino acids 348–597), BPC (amino acids 598–854), BR (amino acids 446–633), KBR (amino acids 446–533) and KBRC (amino acids 446–854). These fragments were tagged with the FLAG or HA sequence at the 3′ end and cloned into pcDNA3.1(+). The deletion mutants of kendrin were constructed by polymerase chain reaction (PCR) using human cDNA as a template and tagged with FLAG at the 5′ end cloned into pcDNA3.1(+): DBR-PACT (amino acids 2913–3232) and PACT (amino acids 3105–3232). These fragments were tagged with the FLAG sequence at the 5′ end and cloned into pcDNA3.1(+).

Antibodies. Polyclonal anti-kendrin antibody was used for the Western blot analysis and immunocytochemistry, and was the kind gift of M. Takahashi and Y. Ono, Kobe University. Polyclonal anti-pericentrin antibody (Covance, Richmond, CA) was used for immunoprecipitation in HEK293T cells. The following antibodies were also used: monoclonal and polyclonal anti-FLAG, monoclonal anti-HA, and monoclonal anti-acetylated tubulin (Sigma–Aldrich, St. Louis, MO) antibodies. The secondary antibodies used were Alexa Fluor 546-conjugated goat anti-mouse or rabbit IgG, Alexa Fluor 488-conjugated goat anti-mouse or rabbit IgG antibody (Molecular Probes Inc., Eugene, OR), and horseradish peroxidase (HRP)-conjugated horse anti-mouse or rabbit IgG antibody (Cell Signaling Tech., Beverly, MA).

Cell culture and transfection. HEK293T and COS-7 cells were cultured in DMEM containing 10% fetal bovine serum (FBS). SK-N-SH cells were cultured in alpha MEM containing 10% FBS. For transient transfection of cells, Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for HEK293T, SK-N-SH, and COS-7 according to the manufacturer's instructions.

Western blot analysis and immunoprecipitation. These experiments were performed as described previously [17].

Immunocytochemistry. Cells were fixed with 4% paraformaldehyde for 20 min at 4 °C or ice cold methanol for 10 min at −20 °C, followed by permeabilization with 0.3% Triton X-100 for 15 min. After blocking with 5% bovine serum albumin, the fixed cells were incubated at 4 °C overnight with the primary antibodies: anti-kendrin (1:500), anti-FLAG (1:1000), and anti-acetylated tubulin (1:500). The secondary antibodies labeled with Alexa Fluor 488 and 546 were then applied for 1 h at room temperature. Cells were observed using a Zeiss LSM510 microscope (Carl Zeiss, Oberkochen, Germany).

Microtubule aster formation assay. COS-7 cells were transfected with the FLAG-tagged DBR-PACT, FLAG-tagged PACT and mock vector, individually. After transfection, cells were incubated on ice for 30 min to depolymerize microtubules. The cold medium was removed and prewarmed medium was added for 1 min. Cells were washed with PBS and then incubated with microtubule stabilizer buffer (80 mM PIPES-KOH pH 6.8, 1 mM EGTA, 1 mM $MgCl_2$ and 0.1% TritonX-100) for 1 min. Cells were fixed with cold methanol for 10 min at −20 °C and immunostained.

Results

The carboxy-terminal region of DISC1 is essential for the DISC1–kendrin interaction

We have previously reported an interaction between overexpressed DISC1 and endogenous kendrin by immunoprecipitation assay in mammalian cells, and further suggested that a short fragment of amino acids 446–533 of DISC1 was essential for the interaction with kendrin by means of a directed yeast two-hybrid

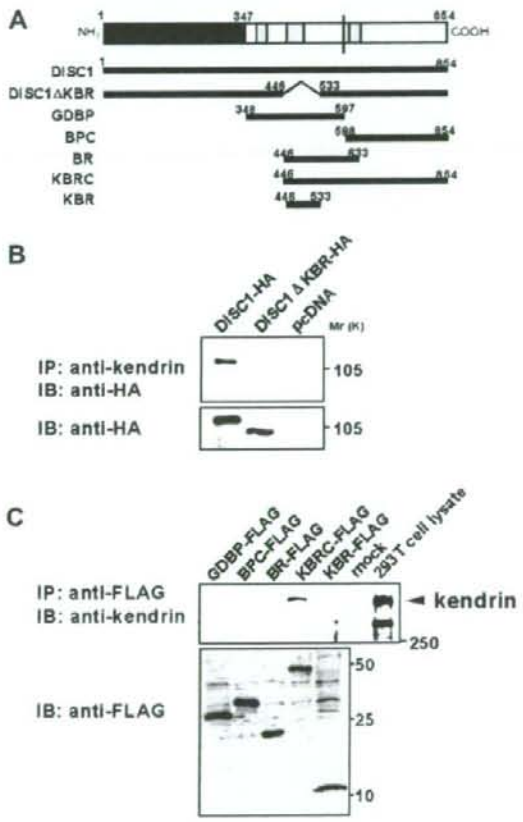

Fig. 1. The carboxy-terminal region of DISC1 is essential for the interaction with kendrin. (A) Structure of deletion mutants of DISC1. The predicted structure of the DISC1 protein is shown in the upper panel. An N-terminal globular region, a carboxy-terminal helical region and three stretches with coiled-coil-forming potential are indicated by black, open and grey rectangles, respectively. The position of the translocation breakpoint is indicated by a vertical line. Various fragments of DISC1 used in this study are shown by closed bars. The numbers indicate the positions of the respective amino acids. (B) HEK 293 cells were transfected with full-length DISC1 (DISC1-HA), a deleted DISC1 that lacks the kendrin-binding region (DISC1 ΔKBR-HA), and empty vector, individually. Immunoprecipitates by anti-kendrin antibody were subjected to Western blot analysis using anti-HA antibody. (C) HEK 293 cells were transfected with various FLAG-tagged fragments of DISC1 shown in (A): GDBP-FLAG, BPC-FLAG, BR-FLAG, KBRC-FLAG and KBR-FLAG. Immunoprecipitates by anti-FLAG antibody were subjected to Western blot analysis using anti-kendrin antibody. KBRC-FLAG interacts with kendrin.

interaction assay [17]. Consistent with the results of our previous study using the yeast two-hybrid assay, the present study confirmed that KBR is critical for the interaction with kendrin by immunoprecipitation assay in HEK293 cells in which kendrin was endogenously expressed. The cells were transiently transfected with the expression vectors for HA-tagged full-length DISC1 (DISC1-HA) and the HA-tagged deletion mutant DISC1 that lacks KBR (DISC1 ΔKBR-HA), individually (Fig. 1A). Cell lysates were prepared, and immunoprecipitates using anti-kendrin antibody were subjected to Western blotting and detected with anti-HA antibody. Endogenous kendrin was coimmunoprecipitated with DISC1-HA, but not with DISC1 ΔKBR-HA (Fig. 1B). These findings indicate that KBR is the binding region of DISC1 to kendrin. Thus, we next examined whether KBR itself could bind to kendrin using an immunoprecipitation assay. For this experiment, we constructed several DISC1 deletion mutants: GDBP-FLAG (amino acids 348–597), BPC-FLAG (amino acids 598–854), BR-FLAG (amino acids 446–633), KBR-FLAG (amino acids 446–533) and KBRC-FLAG (amino acids 446–854) (Fig. 1A). HEK293 cells were transfected with the expression vectors for each deletion mutant. Cell lysates were prepared, and immunoprecipitates using anti-FLAG antibody were subjected to Western blotting and detected with anti-kendrin antibody. Surprisingly, in the immunoprecipitates from cells transfected with KBRC-FLAG, endogenous kendrin was detected. On the other hand, endogenous kendrin was hardly detected in the immunoprecipitates from cells transfected with GDBP-FLAG, BPC-FLAG, BR-FLAG or KBR-FLAG (Fig. 1C). These findings show that KBR is the binding region of DISC1 to kendrin, but this binding to kendrin is enhanced remarkably in the presence of the carboxy-terminal region downstream of KBR. Thus, these results suggest that KBR is required but not sufficient for the interaction, and the carboxy-terminal region of DISC1 is also indispensable for the binding to kendrin.

The carboxy-terminal region of DISC1 is required for the localization of DISC1 to the centrosome

In our previous study, we showed that DISC1 colocalizes with kendrin at the centrosome [17]. As shown in Fig. 1, the KBRC region of DISC1 is essential for the interaction with kendrin. Therefore, we determined which part of DISC1 is indispensable for colocalization of DISC1 with kendrin at the centrosome. For this experiment, human neuroblastoma SK-N-SH cells were transfected with several DISC1 deletion mutants individually: KBRC-FLAG, KBR-FLAG, BR-FLAG, GDBP-FLAG and BPC-FLAG (Fig. 2). Localization of KBRC-FLAG was a diffuse pattern in the cytoplasm but clearly revealed a strong "dot" pattern in the perinucleus (Fig. 2A). The merged image of KBRC-FLAG and kendrin showed that they were colocalized at the centrosome (Fig. 2A). On the other hand, localization of KBR-FLAG showed a diffuse distribution pattern in the nucleus and cytoplasm without strong staining at the centrosome (Fig. 2B). Localization of BR-FLAG and GDBP-FLAG was characterized by a small punctate distribution pattern (Fig. 2C and D). Furthermore, BPC-FLAG exhibited a diffuse distribution in the cytoplasm (Fig. 2E). However, it should be noted that no accumulation of BPC-FLAG was seen at the centrosome. Taken together, these

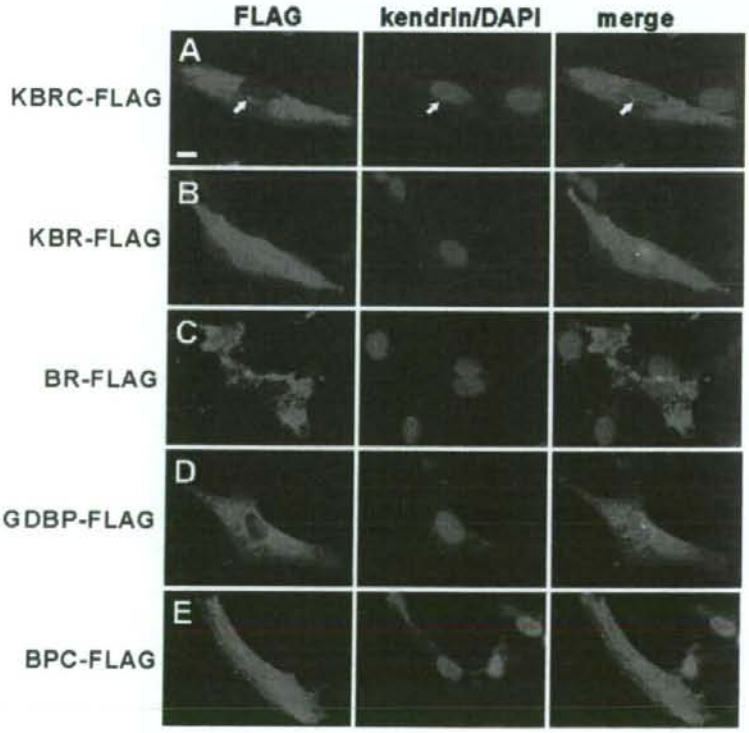

Fig. 2. The carboxy-terminus of DISC1 mediates centrosomal localization. (A–E) Localization of various fragments of FLAG-tagged DISC1. SK-N-SH cells were transiently transfected with KBRC-FLAG (A), KBR-FLAG (B), BR-FLAG (C), GDBP-FLAG (D) and BPC-FLAG (E), respectively. Cells were stained by anti-FLAG antibody (left panels) and anti-kendrin antibody (middle panels). Nuclei were stained blue by DAPI. Merged figures are shown in the right panels. Arrows indicate the colocalization of KBRC-FLAG and kendrin at the centrosome (A). Scale bar, 10 μm.

findings demonstrate that the carboxy-terminal half of the DISC1 protein containing KBR and the downstream region of KBR is necessary and sufficient to target the DISC1 protein to the centrosome, suggesting that this centrosomal localization of DISC1 is mediated by the interaction with kendrin.

Inhibition of DISC1–kendrin interaction perturbs the microtubule network formation

As mentioned above, DISC1 interacts with kendrin at the centrosome. In addition, kendrin has a key role in the microtubule nucleation at the centrosome [20]. Therefore, we analyzed whether the interaction between DISC1 and kendrin might have an influence on the microtubule network formation. To confirm that the DISC1–kendrin interaction is associated with the microtubule organization, we overexpressed the DISC1-binding region of kendrin (DBR) in COS-7 cells. We previously identified amino acids 2918–3035 of kendrin as the DISC1-binding region by yeast two-hybrid screening [17] (Fig. 3A). In addition, to inhibit the DISC1–kendrin interaction specifically at the centrosome, we prepared a FLAG-tagged DBR-PACT (DBR-PACT-FLAG) that includes DBR and the PACT domain (amino acids 3105–3232 of kendrin), a conserved centrosomal targeting motif in CG-NAP and pericentrin [24] (Fig. 3A). We observed the microtubule aster formation in COS cells transfected with either the DBR-PACT-FLAG or PACT-FLAG expression vector (Fig. 3B and C). Mock-transfected cells revealed the microtubule aster formation at the centrosome (Fig. 3E). However, overexpression of DBR-PACT-FLAG resulted in a significant decrease in the percentages of the cells containing the microtubule aster as compared with PACT-FLAG (Fig. 3F). Although previous studies have reported the displacement of endogenous kendrin from centrosomes by overexpression of the PACT domain [25], both DBR-PACT-FLAG and PACT-FLAG were localized at the centrosome without delocalization of endogenous kendrin from the centrosome (data not shown). Furthermore, we analyzed the effect of DISC1 ΔKBR-FLAG, which could not bind to kendrin, on the microtubule aster formation (Fig. 3D). Overexpression of DISC1 ΔKBR-FLAG resulted in a significant decrease in the percentage of cells containing the microtubule aster as compared with mock-transfected cells (Fig. 3F). These results suggest that the DISC1–kendrin interaction is involved in the microtubule organization.

Discussion

The carboxy-terminal region of DISC1 is indispensable for the interaction with kendrin

In the present study, we attempted to confirm the interaction between DISC1 and kendrin by immunoprecipitation assay before analyzing the physiological functions of the DISC1–kendrin interaction at the centrosome. Previously, we suggested that amino acid residues 446–533 of the DISC1 protein would be essential for the

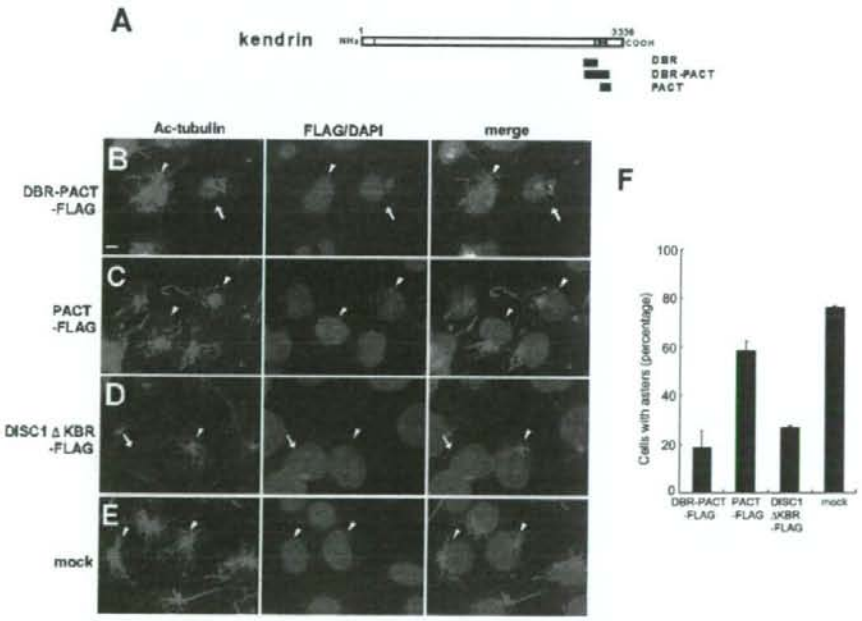

Fig. 3. DISC1–kendrin interaction regulates the microtubule network formation. (A) The structure of the kendrin protein. The region predicted to be a coiled-coil is shown in grey, and various fragments of kendrin, i.e., DBR (DISC1-binding region of kendrin, amino acids 2913–3041), DBR-PACT (amino acids 2913–3232) and PACT (amino acids 3105–3232) are shown with closed bars. (B–E) COS cells were transfected with DBR-PACT-FLAG (B), PACT-FLAG (C), DISC1 ΔKBR-FLAG (D) or mock vector (E). Twenty-four hours after transfection, aster formation was visualized by anti-acetylated tubulin antibody (left panels; green). DBR-PACT, PACT and DISC1 ΔKBR were visualized by anti-FLAG antibody (middle panels; red). Merged images are shown in the right panels. DBR-PACT and PACT were localized to the centrosome (B, C). Normal microtubule aster formation was observed in the PACT-overexpressing cells (C; arrowheads), but not in the DBR-PACT-overexpressing cells (B; arrows) and DISC1 ΔKBR-overexpressing cells (D; arrows). Nuclei were stained blue by DAPI. Scale bar, 10 μm. (F) The percentages of COS cells showing normal microtubule aster formation in DBR-PACT-, PACT-, DISC1 ΔKBR- or mock-overexpressing cells. 58.5% of the PACT-overexpressing cells showed the normal microtubule aster formation. On the other hand, 18.6% of DBR-PACT- and 27.0% of DISC1 ΔKBR-overexpressing cells exhibited the normal microtubule aster formation. At least 50 cells were assessed in each experiment, and columns and vertical bars denote the means ± SEM (triplicate independent experiments). DBR-PACT- and DISC1 ΔKBR-overexpressing cells demonstrated a significant reduction of the normal microtubule aster formation compared with PACT-overexpressing cells ($P < 0.001$, Student's t-test). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

interaction with kendrin, because the yeast two-hybrid analysis indicated that this fragment binds to kendrin [17]. In accordance with these results, binding of KBR-deleted DISC1 to kendrin was not observed in our immunoprecipitation assay, whereas full-length DISC1 could interact with kendrin, suggesting that KBR is indispensable for the binding of DISC1 to kendrin. However, binding between KBR and kendrin was not observed (Fig. 1). We next investigated which region or regions of DISC1 were indispensable for the interaction with kendrin using the deletion mutants of DISC1. As shown in Fig. 1, KBRC, a fragment of DISC1 containing both KBR and the carboxy-terminus, could bind to kendrin. Therefore, the carboxy-terminal region downstream of KBR is essential for the DISC1–kendrin interaction. Furthermore, the KBRC region is also required for DISC1 to target to the centrosome (Fig. 2). Previous study have indicated that the carboxy-terminal region of DISC1 from the breakpoint (i.e., BPC) is required for targeting of DISC1 to the centrosome [26]. Our results further suggest that not only the carboxy-terminal region of DISC1 but also the KBR is indispensable for DISC1 targeting to the centrosome. These results suggest that centrosomal targeting of DISC1 is required for the interaction with kendrin.

The DISC1–kendrin interaction at the centrosome regulates the microtubule network formation

The present study demonstrated that overexpression of the DISC1-binding region of kendrin perturbed the normal distribution of the stabilized microtubule network (Fig. 3). And the overexpression of DISC1 ΔKBR caused the impairment of microtubule aster formation (Fig. 3). These results suggest that the DISC1–kendrin interaction is involved in the normal microtubule network formation. In mammalian cells, two giant centrosomal proteins, kendrin and CG-NAP, have been shown to anchor the γ-tubulin complex to the centrosome, and to play critical roles in the microtubule nucleation [20]. In the carrier of the chromosomal translocation segregating with the mental diseases, the truncated mutant DISC1 protein which lacks the carboxy-terminal region would be produced, or the expression of DISC1 protein would be reduced. In the case of truncated mutant protein expression, this protein would not be able to target to the centrosome and interact with kendrin, which might induce dysfunctions of the microtubule network, such as dysfunctions of organelle transport, protein localization, cell movement and mitotic chromosome segregation. And loss of DISC1 protein expression could lead to the dysfunction of microtubules by disrupting the DISC1–kendrin interaction. In fact, several studies have reported that schizophrenia is associated with abnormality of neuronal development [27–29]. In conclusion, we herein demonstrated that the DISC1–kendrin interaction played a role in neuronal development via the microtubule organization, and our findings cast new light on the etiology of mental disorders.

Acknowledgements

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Central & Peripheral Nervous System

Regulation of pituitary adenylyl cyclase-activating polypeptide (PACAP, ADCYAP1: adenylyl cyclase-activating polypeptide 1) in the treatment of schizophrenia

Shinsuke Matsuzaki† & Masaya Tohyama
Osaka University, The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Graduate School of Medicine, Department of Anatomy and Neuroscience, The 21st Century COE Program, Suita, Osaka, Japan

Background: Deficiency of pituitary adenylyl cyclase-activating polypeptide (PACAP) and its specific receptor, PAC1, causes a schizophrenia-like phenotype in mice. In addition, the relation of the PACAP and PAC1 genes to schizophrenia has been shown by single-nucleotide polymorphism association studies. Furthermore, PACAP is reported to be involved in the function of disrupted-in-schizophrenia 1. *Objective*: To summarize briefly the recent evidence relating the PACAP system and schizophrenia and discuss the application of PACAP to the treatment of schizophrenia. *Results/conclusion*: The regulation of PACAPergic signals is an interesting potential treatment for schizophrenia. Further studies of PACAP signals and the association of PACAP signals with schizophrenia should shed the light on the utility of this approach in the treatment of schizophrenia.

Keywords: Adcyap1, cAMP, DBZ, DISC1, neuropeptide, PACAP, PAC1, psychiatric disease, schizophrenia

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1. Introduction

Pituitary adenylyl cyclase-activating polypeptide (PACAP) was originally isolated as a novel hypothalamic neuropeptide by Arimura's group in 1989, based on its ability to stimulate adenylyl cyclase in rat anterior pituitary cell cultures [1]. PACAP-38 and the C-terminally truncated PACAP-27 are known as biologically active forms and PACAP-27 has an amino acid sequence with 68% shared identity with vasoactive intestinal polypeptide (VIP) and 37% with secretin, indicating that PACAP is a member of the VIP/glucagon/growth hormone-releasing hormone (GHRH)/secretin superfamily. PACAP is present not only in various areas of the central nervous system, including the hypothalamus and other brain regions but also in peripheral tissues, such as testicular germ cells, pituitary gland lobes and the adrenal medulla [2,3]. PACAP has a role in various neurobiological functions, such as neurotransmission and neural plasticity, as well as having neurotrophic effects *via* three heptahelical G-protein-linked receptors, PAC1, vasoactive intestinal peptide/pituitary adenylate cyclase activating peptide receptor 1 (VPAC1) and VPAC2. The PAC1 receptor is specific for PACAP and the two other receptors, VPAC1 and VPAC2, are shared by vasoactive intestinal peptide [2,3]. Previous reports showed that PACAP- and PACAP-specific receptor- (PAC1) deficient mice exhibited prominent behavioral abnormalities, reduced anxiety-like behavior,

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and abnormal social behavior, as well as impairment of hippocampal long-term potentiation (LTP) [4-9]. These observations indicate that PACAP signaling mediated *via* the PAC1 receptor has a critical role in the development and/or functioning of neural pathways and suggests the potential clinical relevance of PACAP signaling dysfunction to neuropsychiatric disorders. Furthermore, the relationship of the PACAP and PACAP-specific receptor genes to schizophrenia was reported in a single-nucleotide polymorphisms (SNPs) association study in a Japanese population [10]. Furthermore, recent studies suggest the involvement of PACAP signals in the neural transmission of abnormalities that are seen in psychotic disorders [11], suggesting that the PACAPergic system is one of the key factors for the pathogenesis of schizophrenia.

Schizophrenia is a devastating psychiatric disorder with a lifetime prevalence of about 1% of the population worldwide, and it commonly has a chronic course. The underlying mechanisms are still largely unknown but a growing body of evidence suggests that schizophrenia is a multifactorial disorder influenced by genetic, neurodevelopmental, and social factors [12-17]. Disrupted-in-schizophrenia 1 (DISC1) has been identified as a potential susceptibility gene for major psychiatric disorders. Disruption of this gene by a balanced (1;11) (q42.1;q14.3) translocation results in a predicted C-terminal truncation of the open reading frame, and this anomaly is segregated with schizophrenia, bipolar affective disorder and recurrent major depression in a large Scottish family [18,19]. In addition, a frameshift mutation of DISC1 has been identified in an American family with schizophrenia and schizoaffective disorder [20], while the association of the single nucleotide polymorphisms of DISC1 with schizophrenia, schizoaffective disorder and bipolar disorder has also been suggested [21]. Recent accumulating studies show that DISC1 plays an important role in neural development in cooperation with binding partners, such as Nudel, lissencephaly 1 (Lis1), fasciculation and elongation protein zeta-1 (FEZ1), Kendrin, phosphodiesterase 4B (PDE4B), and so on [22-29]. In our recent studies, we reported a new DISC1 interacting partner, DISC1-binding zinc-finger protein (DBZ), and showed that PACAP signaling *via* PAC1 regulates the interaction between DISC1 and DBZ [11], suggesting that the abnormality of DISC1, DBZ or PACAP-PAC1 signaling causes immature neural developments which result in susceptibility to psychiatric diseases.

In this article, we review the involvement of PACAP signaling in mental disorders, focusing on schizophrenia, in terms of the neurotransmission and the DISC1–DBZ interaction regulated by the PACAPergic signal.

2. Schizophrenia and new treatments for schizophrenia

Schizophrenia is a chronic psychotic illness with a lifetime prevalence of about 1% of the population worldwide, which shows overt psychosis striking typically during late adolescence and causing severe lifelong disability [30]. Family studies show that the risk of schizophrenia is significantly higher in families of schizophrenia probands than in the general population or in families of other affective disorder probands [31]. Since the concordance rate in monozygotic twins is not 100%, genetic background alone does not induce schizophrenia. However, the monozygotic concordance is higher than dizygotic concordance [32]. Therefore, it is certain that genetic background plays an important role in schizophrenia. Genetic linkage studies identified several candidate loci on the genome on the following chromosomes: 1q21 – 44, 5q22 – 31, 6p24 – 22, 8p22 – 21, 10p15 – 11, 13q14 – 32, and 22q11 – 13 [30]. Subsequent studies have identified several candidate genes for schizophrenia such as DISC1, dysbindin, catechol-*O*-methyltransferase (COMT), neureglin 1, G72, Regulator of G protein signalling 4 (RGS4), and so on [30-47].

The schizophrenic patients show three major symptoms: positive psychotic symptoms, disorganization in thought and behavior, and negative symptoms [48], showing that schizophrenia is multifunctional in origin. In addition, several functional impairments in cognitive functions are known to be symptoms of schizophrenia [49]. These impairments are used as essential diagnostic criteria in the Diagnostic and Statistical Manual IV (DSM-IV). Based on accumulating studies of the symptoms of schizophrenia, several hypotheses based on abnormal neurotransmission are supported, such as the dopamine hypothesis, which is based on hyperactive dopaminergic signal transduction, the glutamate hypothesis, which is based on hypofunction of glutamatergic signaling, and the serotonin hypothesis, which is based on hypofunction of serotonergic signaling [50-56] Furthermore, studies of brain imaging have elucidated significant changes in neural transmission in the schizophrenic brain [57-60].

New treatments for schizophrenia have been developed based on the finding that dopamine-enhancing drugs mimic psychotic symptoms. In fact, dopamine D2 receptor blockers, such as chlorpromazine, are effective for the treatment of schizophrenia, particularly for the positive psychotic symptoms. Dopamine D2 receptor blockers decrease psychotic symptoms, such as hallucinations, delusions and agitation [61-65]. However, they cause some side effects: the extrapyramidal side effects such as Parkinsonism and abnormality of the endocrine system, such as hyperprolactinemia, and they have little effect on the negative symptoms [66-67]. Thus, the 5-hydroxytryptamine 5-HT_2 receptor became the center of attention for the next target of schizophrenia therapy and serotonin-dopamine antagonists (SDA), which inhibit both dopamine D2 receptor and 5-HT_2 receptor [68]. The SDA has the advantage of reduced side effects and improvement of several negative symptoms compared with the dopamine D2 receptor inhibitor [69-74]. Now serotonin $5\text{-HT}_1\text{A}$ receptor partial agonists, selective

agonists for metabotropic glutamate 2/3 receptors and so on, are in the spotlight as the next targets [75-79]. It is well known that the inhibition of glutamate transmission induces positive and negative psychotic symptoms. Accordingly, selective agonists for metabotropic glutamate 2/3 receptors produced significant improvements in both positive and negative symptoms of schizophrenia compared with placebo [74]. As mentioned above, the regulation of the glutamatergic system, as well as dopaminergic and serotnergic systems, is an important target for the treatment of schizophrenia (**Figure 1**).

Another hypothesis is that there is an impaired development of neural networks in the brains of schizophrenia patients. Recent advances in imaging technology (such as functional MRI (fMRI) and diffusion tensor imaging (DTI)) have enabled investigators to move beyond measures of isolated regional abnormalities, and instead begin to explore the function and structure of the interconnected neural networks that are implicated in schizophrenia. The most consistent structural abnormalities found in schizophrenia include lateral and third ventricular enlargement; medial temporal lobe (hippocampal formation, subiculum, parahippocampal gyrus) volume reductions; and superior temporal gyrus volume reductions, particularly on the left, and several other abnormalities in other parts of the brain have been reported [80-82]. In addition, abnormal brain structure may be detectable *via* MRI prior to the onset of psychotic symptoms [83], and these anomalous late neurodevelopmental processes may interact with other environmental factors associated with the onset of psychosis (e.g., stress, substance abuse), which together have neuroprogressive sequelae that may be neurodegenerative [84,85]. These reports suggest the involvement of abnormal neurodevelopment in schizophrenia.

3. PACAP and PAC1 are key susceptibility factors for major mental illnesses

3.1 Genetic association of PACAP and PAC1 to major mental illnesses

Many studies reported that susceptibility genes may be present on chromosome 18p where the PACAP gene is located [86-91]. A possible linkage of certain psychiatric diseases with the PACAP gene Adcyap1 has been suggested. Studies in two related patients with a partial trisomy 18p revealed three copies of the PACAP gene and elevated plasma PACAP levels. These patients suffered from severe mental retardation and hematological abnormalities, although whether the former defect is a consequence of PACAP overexpression remains to be determined [92]. Fine-scale mapping of a locus for severe bipolar mood disorder on chromosome 18p11.3 suggests that Adcyap1, residing at 18p11.32, is located close to a bipolar disorder risk locus [92-94]. In addition, recent studies reported that genetic variants of the PACAP and PAC1 genes are associated with schizophrenia and that the risk SNP of the PACAP gene could be associated with reduced hippocampal volume and poorer memory performance [10]. These reports support the association of the PACAPergic system with psychiatric disorders, especially bipolar disorder and schizophrenia.

3.2 Abnormal behaviors related to mental illnesses in PACAP-deficient mice and the effects of the defect or overexpression of PAC1 on mice

The suggestion mentioned above, which is based on data from humans, is also supported by data obtained from genetically engineered animals. PACAP-deficient mice displayed the abnormal behaviors, such as significantly increased locomotor activity with minimal habituation to the environment, explosive jumping behavior, increased exploratory behavior, and less time engaged in licking and grooming behavior in a novel environment [4]. Furthermore, PACAP-deficient mice display prepulse inhibition (PPI) deficits and impairments of PPI hippocampal LTP [4-6]. In addition, hyperactivity in PACAP knockout mice was abolished by an atypical antipsychotic drug, haloperidol (D_2 antagonist). Risperidone, a combined D2 and $5HT_{2A}$ receptor antagonist, reversed the hyperactivity and diminished PPI in PACAP KO mice to the level observed in wild type mice [10], suggesting that the abnormal behaviors in PACAP KO mice are schizophrenia-like phenotypes in rodents. In addition, the jumping behavior is suppressed by drugs that elevate extracellular serotonin, such as the selective serotonin re-uptake inhibitors (see Section 3.3) [95].

PAC1 receptor-deficient mice also exhibited an increase in locomotor activity, reduced anxiety-like behavior, and abnormal social behavior, as well as impairment of hippocampal LTP [96-98]. PAC1-overexpression displayed strikingly similar phenotypes to PAC1 knockout mice, hydrocephalus-related phenotypes [99]. Furthermore, chronic treatment with PCP, which induces positive symptoms, negative symptoms and cognitive impairments similar to those seen in patients with schizophrenia, reduced the mRNA expression of PACAP and PAC1 in the frontal cortex (**Figure 2**) [10].

These observations indicate that PACAP signaling, mediated *via* the PAC1 receptor, is associated with the pathophysiology of schizophrenia and has a critical role in the development and/or functioning of neural pathways and also indicate a potential role of PACAP signaling dysfunction in neuropsychiatric disorders.

3.3 Association of the PACAP–PAC1 receptor system with other neurotransmitter systems such as the dopaminergic, glutamatergic and serotonergic systems (Figure 2)

PACAP has been shown to increase tyrosine hydroxylase (TH) protein activity and mRNA levels *in vivo* and *in vitro* [100,101]. Intracerebroventricular injection of PACAP increased dopamine release in the hypothalamus in sheep [102]. Hyperlocomotion and jumping behavior but

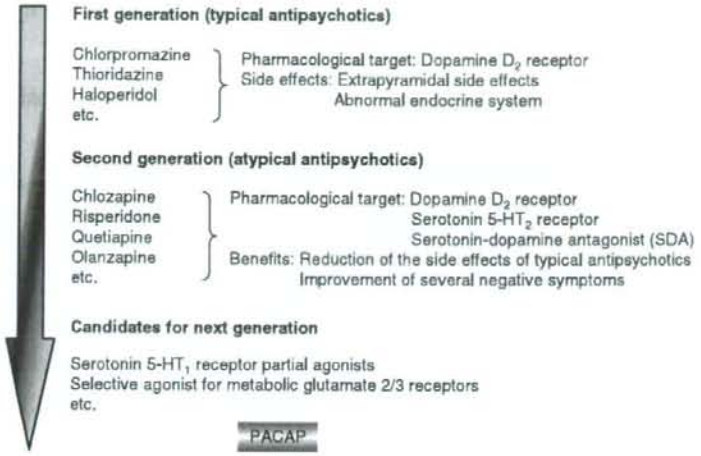

Figure 1. Development of the treatments for schizophrenia. First, typical antipsychotics were developed as a treatment for schizophrenia. Next the 5-hydroxytryptamine (5-HT_2) receptor became the center of attention for schizophrenia therapy because atypical antipsychotics has the advantage of reduced side effects and improvement of several negative symptoms compared with typical antipsychotics. Now the regulation of glutamate transmission is one of the important targets of the treatment for schizophrenia. Regulation of PACAP signals might be a potential treatment for schizophrenia.

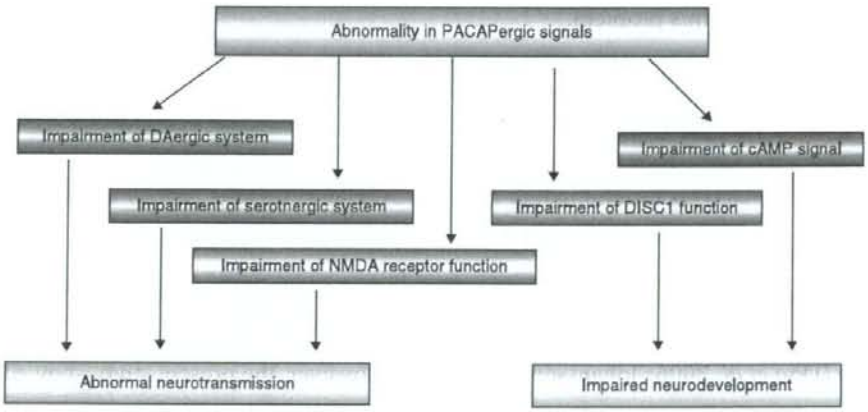

Figure 2. Impairment of PACAP signals and schizophrenia Impaired PACAP signals resulted in several abnormal phenotypes *in vivo* and *in vitro*. The abnormalities caused by the impaired PACAPergic system have close relationship to several hypotheses regarding the pathology for schizophrenia, such as abnormal neurotransmission and impaired neurodevelopment.
DISC1: Disrupted-in-schizophrenia 1.

not deficits in PPI, in PACAP deficient mice were attenuated effectively by haloperidol (a D2 antagonist) [4,5]. Furthermore, the selective serotonin re-uptake inhibitor, fluoxetine, as well as the serotonin precursor, 5-hydroxytryptophan, suppress jumping behavior. Risperidone, a combined D2 and 5-HT_{2A} receptor antagonist, reversed both of these abnormalities in $PACAP^{-/-}$ mice [10]. These accumulating studies indicate the presence of an interaction between the PACAP system and the dopaminergic and serotonergic systems. On the other hand, several studies have shown that PACAP can potentiate NMDA receptor functions [103-105] Furthermore, Mabuchi *et al.* showed that PACAP deficient mice do not exhibit inflammatory or neuropathic pain, and that PACAP is required for functional coupling of neuronal nitric oxide synthase to NMDA receptors in the spinal cord for chronic pain to occur [106]. These reports suggest that PACAP signals also involve the glutamatergic system *via* NMDA. The NMDA antagonist, MK-801, induces similar behavior to PACAP knockout [107]. Furthermore, chronic treatment with PCP, which induces positive symptoms, negative symptoms and cognitive impairments, similar to those seen in patients with schizophrenia, reduced the mRNA expression of PACAP and PAC1 in the frontal cortex [10]. These results suggest an association between the PACAP and glutamatergic system. As mentioned in Section 2, the regulation of the glutamatergic system is a potential target for treatment. The regulation of PACAP may shed light on potential schizophrenia treatments.

4. PACAP-PAC1 signaling and mental disease

4.1 DISC1 and schizophrenia

DISC1 has been identified as a potential susceptibility gene for major psychiatric disorders [18,19]. A balanced translocation of DISC1 segregated with schizophrenia and affective mental disorders. Therefore, it is likely that the function of the molecular complex composed of DISC1 and the binding proteins, which bind to the translocation site of DISC1, is disturbed in schizophrenic patients. Recently, using the yeast two-hybrid method, we have identified three molecules that bind to the domain of DISC1 including the translocation site, namely FEZ1, DBZ and Kendrin [11,25,26]. In addition, other authors have identified interacting partners of DISC1, such as Nudel, Lis1 and PDE4B [22-24,27-29] The function of the DISC1–FEZ1 interaction was explored by us [25]. DISC1 and FEZ1 were found to be co-localized in growth cones in association with F-actin in both SK-N-SH cells and cultured hippocampal neurons [11]. The interaction of DISC1 with FEZ1 was enhanced markedly along with neurite extension in PC12 cells by nerve growth factor (NGF) stimulation [11,108]. An association between single nucleotide polymorphisms of the *FEZ1* gene and schizophrenia has also been suggested in a Japanese population [109]. In the report, we showed the importance of the DISC1–FEZ1 interaction in neural development and supported the hypothesis that impaired brain development causes schizophrenia. We also found involvement of PACAP in neural development *via* the regulation of DISC1–DBZ interaction [11]. The following is a detailed description of the DISC1–DBZ interaction (see Section 4.2).

4.2 PACAP–PAC1 signaling regulates the DISC1–DBZ interaction (Figure 3)

DBZ encodes a putative 407 amino acid protein without significant homology to any other known protein and is expressed exclusively in the brain. DBZ is expressed in neurons, not in glial cells. DBZ is colocalized with DISC1 diffusely in the cytoplasm. We searched for molecules that influence the DISC1–DBZ interaction and found that PACAP has a marked influence on the endogenous DISC1–DBZ interaction. The co-immunoprecipitation of DISC1 with DBZ in lysates is reduced by 80% 1 h after treatment of PC12 cells with PACAP. Thereafter, the DISC1–DBZ interaction increases gradually, returning to the control level by 24 h after treatment. Thus, PACAP stimulation caused a transient dissociation between DISC1 and DBZ [11]. To investigate the physiological role of the DISC1–DBZ interaction, the effects of enhanced DISC1–DBZ binding on PC12 cells was examined in the PC12 cells expressing DISC1–haemagglutinin (HA) and DBZ–green fluorescent protein (GFP). After the DISC1/DBZ overexpressing cells were treated with PACAP for 48 h, the number of neurite-bearing cells was counted. Overexpression of both DBZ and DISC1, which enhances the DISC1–DBZ interaction, resulted in a significant decrease in the number of neurite-bearing PC12 cells, whereas overexpression of either DBZ or DISC1 alone did not alter the number of neurite-bearing cells significantly. No significant changes in neurite length or the number of neurites per cell were observed in either the mock- or DISC1-HA-transfected PC12 cells, irrespective of the expression of DBZ. Furthermore, we investigated the effect of the inhibition of the dissociation between DISC1 and DBZ on neurite outgrowth to confirm the role of DBZ dissociated from DISC1 under PACAP stimulation. Overexpression of the DISC1 binding domain of DBZ inhibits the neurite outgrowth of PACAP-treated PC12 cells and primary cultured neurons without any significant change in apoptosis of gene-transfected PC12 cells. These findings show that the DISC1–DBZ interaction has an important role in neurite growth and suggest that one function of DBZ is as a negative regulator of DISC1 function. Furthermore, we showed that the PAC1 inhibitor, which inhibits the effect of PACAP on neurite outgrowth, reduced the dissociation between DISC1 and DBZ caused by PACAP stimulation (Matsuzaki *et al.*, unpublished). Thus, in the normal brain, the dissociation of the DISC1–DBZ interaction by PACAP *via* the PAC1 receptor should cause neurite extension, and the subsequent increase in the DISC1–DBZ interaction may inhibit neurite outgrowth and neural circuit formation (Figure 3A). In schizophrenia in DISC1 translocation

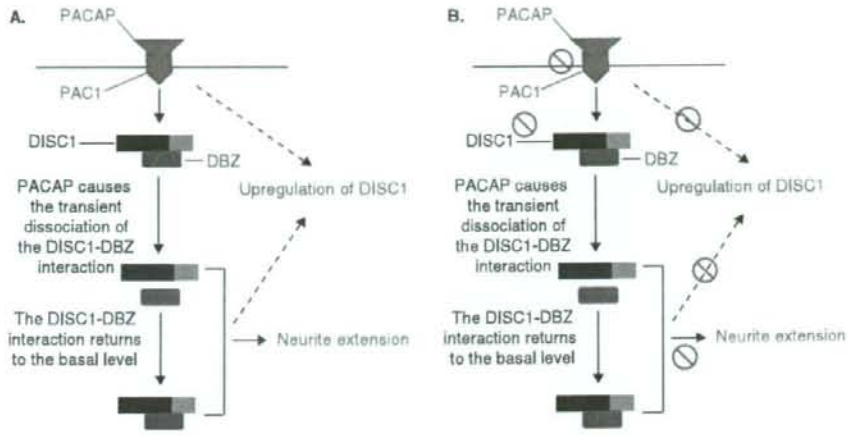

Figure 3. Regulation of DISC1 function via PACAP signal PACAP signal via PAC1 causes the transient dissociation of DISC1–DBZ interaction and results in neurite outgrowth. An abnormality of PACAP, PAC1 or DISC1 should cause the inhibition of neurite extension.

DBZ: DISC1 binding zinc-finger protein; DISC1: Disrupted-in-schizophrenia 1; PAC1: PACAP specific receptor; PACAP: Pituitary adenylyl cyclase-activating polypeptide.

carriers, DBZ cannot bind to DISC1 because of the translocation. Therefore, neurite growth by dissociation of the DISC1–DBZ interaction by PACAP is inhibited and neural circuit formation remains immature (**Figure 3B**). This event seems to occur in the granule cells of the dentate gyrus because these cells express both DBZ and PAC1 in comparisons of the distribution of neurons expressing DBZ mRNA with those expressing the PACAP receptor PAC1 mRNA (Matsuzaki *et al.* unpublished). Furthermore, PACAP in the granule cells of the dentate gyrus is supplied by the PACAP neurons in the entorhinal cortex through perforating fibers. Thus, disruption of the DISC1–DBZ interaction by abnormalities in DISC1, DBZ, or PACAP–PAC1 signaling causes immature neural circuit formation in the granule cell layer of the dentate gyrus and abnormalities in PACAP-PAC1 signaling may participate in the immature neural development of schizophrenic brains.

4.3 Molecular mechanism of upregulation of adenosine 3′,5′-monophosphate (cAMP) production by the binding of PACAP to the PAC1 receptor

PACAP stimulates cAMP and a recent paper suggests the involvement of the PAC1 receptor in the enhancement of cAMP generation [110,111]. Furthermore, patients with a trisomy 18p, who show elevated PACAP concentration in plasma, have upregulation of cAMP levels [92]. These reports suggest that the PACAP–PAC1 signal is of importance in the enhancement of cAMP. Recently, Millar *et al.* reported the regulation of cAMP level *via* the DISC1–PDE4B interaction as well as a close association of PDE4B with schizophrenia [23]. The regulation system involves the elevation of cellular cAMP, which leads to dissociation of PDE4B, one of the inactivators of cAMP, from DISC1, and an increase in PDE4B activity. Furthermore, lower activity of PDE4B was identified in the DISC1 mutant mice, which exhibit schizophrenia-like behavior [112]. A PDE4B haplotype alters the genetic risk of schizophrenia in a Scottish population which is consistent with the known participation of this gene in biological processes associated with mental illness [113]. These findings showed that the upregulated cAMP level by binding of PACAP to PAC1 might be caused by an increase in the DISC1–PDE4B interaction and return to the basal level via the dissociation of DISC1–PDE4B interaction.

Many reports support the involvement of an abnormal cAMP level in mental disease and involvement of abnormalities in components of cAMP signaling in schizophrenia [114-117]. Lower levels of Gi were reported in schizophrenic patients [118], whereas the cAMP response to forskolin, as well as the binding of forskolin to adenylyl cyclase, were found to be elevated significantly in schizophrenic patients [119-120]. These alterations may have associated effects on PKA, a crucial component of cAMP signaling which mediates most of the actions of cAMP, including the regulation of gene expression [121]. An increase in cAMP binding to PKA was reported in schizophrenic patients [118].

As mentioned above, PACAP–PAC1 signals not only dissociate the DISC1–DBZ interaction, which cause neurite outgrowth but also increase the cAMP level. After the dissociation of the DISC1–DBZ interaction, the DISC1–PDE4B interaction might be induced and result

in the upregulation of cAMP, suggesting a strong involvement of PACAP–PAC1 signals in the pathogenesis of schizophrenia. Thus, the regulation of the PACAPergic system, especially *via* the PAC1 receptor, might be a new target for schizophrenia treatment based on the impaired neurodevelopment hypothesis (Figure 2).

5. Expert opinion. Regulation of PACAP signaling as a schizophrenia therapy

Schizophrenia is a disorder caused by the complex interplay of genetic and environmental factors, and the phenotype of the symptoms is quite variable with positive, negative and cognitive symptoms. These things make the treatment of schizophrenia difficult. However, accumulating studies have elucidated that an abnormal neural network formation and abnormal neural transmission is common to schizophrenia. Thus, it is necessary to improve these abnormalities to treat schizophrenia. As described in Sections 2 – 4, PACAP, one of the susceptibility genes of schizophrenia, is involved in both neural transmission and neural development suggesting that the regulation of PACAP might be one of the keys to the treatment of schizophrenia (Figure 2).

As mentioned before, hypofunction of PACAPergic signals is involved in schizophrenia. With hypofunction of the PACAP system, dopaminergic and serotonergic systems are activated, while the glutamergic system is inactivated. Thus, the PACAP system is present upstream of regulation of dopaminergic, serotonergic and glutamergic transmission, and regulation of the PACAP signal pathway is much more effective and selective for the treatment of schizophrenia than that of regulation of the dopamine, serotonin and glutamate systems. In addition, Shintani *et al.* showed that the alteration observed in PACAP knockout mice recovered by the addition of transient PACAP [95] and transient PACAP stimulation of dorsal root ganglion (DRG) neurons affects the NMDA receptor response by increasing the stability of the NMDA receptor via the functional coupling of neuronal nitric oxide synthase to the NMDA receptors [106]. These data suggest that the abnormal phenotype of PACAP- and PAC1-deficient mice contributes not only to the abnormal neural circuit via the impairment of PACAPergic signals but also by direct effects of PACAPergic signals on neural transmission. Thus, PACAP may be a therapeutic effector for the symptoms of schizophrenia via the abnormal transmissions. Hyper-dopaminergic signals might be ameliorated by the inhibition of PACAP signals and hypo-glutamaergic or hypo-serotonergic signals might be ameliorated by the upregulation of PACAP via the upregulation of the NMDA receptor or the upregulation of the serotonergic system. Hence, the regulation of PACAP signals may treat abnormal neural transmission observed in schizophrenic brains in multiple ways. In addition, PACAP–PAC1 signal also plays an important role in neural development and the impairments in PACAPergic signals cause abnormal brain development (see Sections 4.2 and 4.3), whereas PACAP is thought to be one of the risk factors for schizophrenia from the standpoint of the hypothesis that abnormal neural development is one of the risks for schizophrenia.

As shown here, the regulation of PACAPergic signals as the therapy for schizophrenia should improve not only dopaminergic, serotonergic and glutamergic transmission but also neural development. In comparison with traditional treatment strategies, PACAP therapy has this advantage. Thus, the regulation of PACAP or PACAPergic signals has promising therapeutic efficacy for schizophrenia.

Is it possible to regulate the PACAPergic signals in human? A way to regulate the PACAP signals in humans has not established yet, but several studies suggest the potential for regulating PACAP levels. A previous study showed that lithium stimulation, one of the therapies for bipolar disorder, caused the upregulation of PACAP levels [122]. This result might indicate a method for upregulation of PACAP on psychiatric diseases. On the other hand, previous studies indicate that the major isoforms of PACAP, (1 – 27) and (1 – 38), are degraded by the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV) to form PACAP(3 – 27) and (3 – 38) [123] and that DPP-IV degrade PACAP(1 – 27) to PACAP(3 – 27), to PACAP(5 – 27) and finally to PACAP(6 – 27), which has been suggested to antagonize the actions of PACAP in the pancreatic cell [124]. These findings indicate that the inhibition of DPP-IV should be effective for upregulating PACAP levels.

Lastly, we discuss the side effects of PACAPergic-based therapies for schizophrenia because we cannot ignore these. As mentioned above, PACAP–PAC1 signaling also affects DISC1 function via alteration of the DISC1–DBZ interaction [11]. We suggest that DISC1 interacts with other partners, such as FEZ1, to function at several biological levels after dissociation from DBZ. In addition, we have reported that PACAP stimulation causes the upregulation of DISC1 [11]. Thus, continuous inhibition of PACAP signaling might cause the downregulation of DISC1 function because of the downregulation of DISC1 expression. Furthermore, continuous inhibition should cause several side effects since PACAP- and PAC1-knockout mice show several abnormalities [4-6,10,96-98]. In addition, the inhibition of PACAP signaling should inhibit cAMP activity directly and indirectly *via* the downregulation of the DISC1–PDE4B interaction. Conversely, continuous stimulation by PACAP should affect DISC1 function and might cause the upregulation of DISC1. Kamiya *et al.* showed that overexpression of DISC1 causes the hyperformation of the centrosome [125]. Patients with trisomy 18p, whose PACAP level is elevated, show mental retardation, and PAC1-transgenic mice showed abnormal neural development [92,103]. These results suggest that continuous stimulation by PACAP causes problems in neurodevelopment via the upregulation of cAMP and DISC1 function. Thus, moderate PACAP stimulation is that key to

maintaining normal neural network systems. Summarizing these data, the pulse inhibition of PACAP might be effective in patients with mental disorders or schizophrenia via the hyper PACAPergic system and the pulse stimulation of PACAP might be effective in patients with mental disorders or schizophrenia via the hypo PACAPergic system.

In conclusion, the regulation of PACAPergic signals is an interesting potential treatment for schizophrenia. Further studies of PACAP signals and the association of PACAP signals with schizophrenia should shed light on the utility of this approach in the treatment of schizophrenia.

Declaration of interest

The authors declare no conflicts of interest and have received no payment for the preparation of this manuscript.

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