200835071A

厚生労働科学研究費補助金

地域医療基盤開発推進研究事業

抑肝散の示す精神疾患周辺行動改善に対する科学的検証に関する研究

平成２０年度　総括研究報告書

研究代表者　遠山正彌

平成２１（２００９）年　３月

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（総括）研究報告書

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研究要旨
本研究により抑肝散、とりわけセンキュウはアルツハイマー病ゆらいの神経細胞死抑制作用を有しており、このことはアルツハイマー病の神経細胞死を抑える治療薬、又早期より服用することで予防薬として極めて有力な候補であることを示した。
また抑肝散、とりわけチョウトウコウの 5HT 受容体に対する作用からかんがみると、統合失調症の新規の治療薬としても有効であると思われる。

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A. 研究目的
本研究の研究目標は臨床的に効果が認められている漢方薬、抑肝散の BPSD・精神疾患（統合失調症を中心とした）改善効果を科学的に立証し、抑肝散の作用機序を解明することにある。また各種病態モデルによる検討から抑肝散が最も効果的な精神疾患を明らかとする。

B. 研究方法

1）抑肝散のアルツハイマー病神経細胞死に対する効果とその分子機序

我々はアルツハイマー病による神経細胞死が小胞体の蛋白質品質管理機構の破綻によることを世界に先駆けて報告した。種々の小胞体ストレスは小胞体内に unfolded protein を蓄積する。正常細胞ではこの不良蛋白質排除機構が働くがアルツハイマー病患者の神経細胞では排除機構が作動せず不良蛋白質が小胞体内に過剰蓄積してカスペース４を活性化、細胞死に至る。本研究では神経芽細胞由来の SK-N-SH 細胞を使用する。SK-N-SH 細胞に種々の小胞体ストレスを負荷する（低酸素、Tunicamycine, Thapsigaldin など)。正常細胞では細胞死を起こす細胞は少数で、多くの細胞では小胞体シャペロンの GRP78 が誘導され不良蛋白質の正常化を図り、カスペース４の活性化も低レベルである。しかしアルツハイマー病脳で発現するプレセニリン１や２の変異体を強制発現した細胞では GRP78 の誘導が極めて低レベルで、カスペース４が活性化される。これらの細胞に抑肝散を投与し、特にプレセニリン１変異体発現細胞での GRP78 発現、カスペース４の変動を検討した。

また抑肝散構成７種の生薬のうちどの生薬が最も有効な作用を有するかをも明らかにすることを試みた

2）抑肝散の統合失調症に対する効果とその分子機序

名古屋私立大学医学部の島田教授のグループと共同で検索した。統合失調症の発現に関与する 5HT(セロトニン) 1A,2C,D（ドーパミン) D1,D2 受容体に対する作用を検討した。前者はカルシウムの変動により測定できるが、後２者は cAMP の変動がマーカーとなるため、４者の作用を同時に観察することはできない。そこでキメラ蛋白を用いたアッセイ系を確立し４者が同時に測定できるシステムを構築した。この系を用いて抑肝散の各種受容体に対する作用を検討し、さらに抑肝散のどの構成生薬が最も有効な効果を発揮するかを明らかとした。

（倫理面への配慮）
本実験では培養細胞を用いたため倫理面での特別な配慮は必要ない。

C.研究成果

I）抑肝散とその有効成分はアルツハイマー病の治療薬、予防薬として有効であるか？

抑肝散は小胞体ストレスによる神経細胞死を救済する

図１に示すごとく抑肝散は低酸素負荷（小胞体ストレスの一種）による神経細胞死を抑制した。低酸素負荷を加えると図左に示すように多くの神経細胞が細胞死を起こす（赤色)。しかしながら抑肝散を加えると神経細胞死をおこす細胞が激減した（図１右)。アルツハイマー病の原因遺伝子 PS1 の変異が神経細胞に発現していると小胞体ヌトレスによる神経細胞死は非発現群と比べると著しく増加する。しかしながら抑肝散投与群では神経細胞死を著しく抑える。
又抑肝散投与群では GRP78 発現が非投与群に比し著しく増加していた。逆にカスペース４の活性化が抑えられていた。同様な効果は抑肝散構成生薬の一つであるセンキュウで認められたが、他の生薬成分では殆ど認められない。またセンキュウは培養細胞に対し毒性を示さなかった。

II）抑肝散は統合失調症の有力な治療薬か？
以上の結果は抑肝散は５HT1Aにはagonistとして、5HT2A,2Cに対してはantagonistとして強力な作用を有していることが判明した（図２）。現在ドーパミン受容体に対する作用を検討中である。又抑肝散のこの作用はチョウトウコウガに名手いることが明らかとなった。

E. 結論
本研究により抑肝散、とりわけセンキュウはアルツハイマー病ゆらいの神経細胞死抑制作用を有しており、このことはアルツハイマー病の神経細胞死を抑える治療薬、又早期より服用することで予防薬として極めて有力な候補であることを示す。また抑肝散、とりわけチョウトウコウの5HT受容体に対する作用からかんがみると、統合失調症の新規の治療薬としても有効であると思われる。

F. 健康危険情報
特になし。

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H. 知的財産権の出願・登録状況（予定を含む。）
１．特許取得
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２．実用新案登録
３．その他

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Biochemical and Biophysical Research Communications 379 (2009) 191–195

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dysbindin engages in c-Jun N-terminal kinase activity and cytoskeletal organization

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ARTICLE INFO

Article history:
Received 2 December 2008
Available online 16 December 2008

Keywords:
Dysbindin
Schizophrenia
c-Jun N-terminal kinase
Actin cytoskeleton

ABSTRACT

A number of reports have provided genetic evidence for an association between the DTNBP1 gene (coding dysbindin) and schizophrenia. In addition, sandy mice, which harbor a deletion in the DTNBP1 gene and lack dysbindin, display behavioral abnormalities suggestive of an association with schizophrenia. However, the mechanism by which the loss of dysbindin induces schizophrenia-like behaviors remains unclear. Here, we report that small interfering RNA-mediated knockdown of dysbindin resulted in the aberrant organization of actin cytoskeleton in SH-SY5Y cells. Furthermore, we show that morphological abnormalities of the actin cytoskeleton were similarly observed in growth cones of cultured hippocampal neurons derived from sandy mice. Moreover, we report a significant correlation between dysbindin expression level and the phosphorylation level of c-Jun N-terminal kinase (JNK), which is implicated in the regulation of cytoskeletal organization. These findings suggest that dysbindin plays a key role in coordinating JNK signaling and actin cytoskeleton required for neural development.

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Schizophrenia is a heritable mental disease that devastates about 1% of the population worldwide, affecting their perception, emotion, and judgment [1]. The DTNBP1 gene (coding dysbindin: dystrobrevin binding protein (1) was identified as a candidate for involvement in schizophrenia [2]. In studies of postmortem brain tissue, patients with schizophrenia had lower dysbindin expression than controls [3–5]. Moreover, long-term treatment with typical or atypical antipsychotics did not alter the mRNA expression levels or protein levels of dysbindin in the mouse frontal cortex and hippocampus [4,6]. Together these findings suggest that decreased dysbindin levels may confer susceptibility to schizophrenia. Sandy (*sdy*) mice that express no dysbindin, owing to a deletion of the DTNBP1 gene showed behavioral abnormalities such as reduced activity, heightened anxiety-like response, and deficits in social interaction, memory, and learning [7–9], which could be endophenotypes of schizophrenia. *sdy* mice also displayed lower levels of dopamine, but not glutamate, in the cerebral cortex, hippocampus, and hypothalamus [8,10].

Dysbindin is known to be widely distributed in the brain and located presynaptically and postsynaptically in the central nervous system [11]. The downregulation of endogenous dysbindin by small interfering RNA (siRNA) reportedly inhibited the release of glutamate from hippocampal cultured neurons and increased the release of dopamine from PC12 cells [12,13]. In addition, *sdy* mice reportedly exhibit defective synaptic structure and function in the hippocampal CA1 neurons [14]. However, the molecular mechanism underlying the effects of dysbindin on synaptogenesis remains elusive. On the other hand, an increasing number of studies have shown that cytoskeletal organization is essential for the dynamics of synaptogenesis [15,16]. Therefore, to examine the effects of low dysbindin levels on cytoskeletal organization, we performed an immunocytochemical analysis using SH-SY5Y cells, which have been used as an *in vitro* model to study neural development. Furthermore, to confirm the influences of dysbindin knockdown *in vivo*, we analyzed *sdy* mice similarly. In addition, we investigated whether alterations in dysbindin expression affect c-Jun N-terminal kinase (JNK) activity, which has been known to phosphorylate many cytoskeletal proteins and regulate neural development [17–20].

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doi:10.1016/j.bbrc.2008.12.017

Materials and methods

Mice. The *sdy* and control DBA/2J mice were originally obtained from the Jackson Laboratory and bred in the Institute of Experimental Animal Sciences, Osaka University Graduate School of Medicine. All the experiments were performed in accordance with our institutional guidelines after obtaining the permission of the Laboratory Animal Committee.

Cell culture. The SH-SY5Y cell line was obtained from the European Collection of Cell Culture (Wiltshire, UK) and maintained according to the manufacturer's protocol. Hippocampal neurons were cultured from E15 mice embryos using the Nerve-cell Culture System (SUMITOMO BAKELITE, Tokyo, Japan). Neurons (2.1×10^5) in MEM (Invitrogen) containing 10% fetal horse serum and antibiotics (50 U/ml penicillin G and 50 μg/ml streptomycin) were plated onto 3.5 cm poly-L-lysine-coated dish. After 4 h of incubation, medium was changed to Neurobasal Medium containing 2% B27 (Invitrogen) and antibiotics.

Plasmid. FLAG-human *dysbindin* (AF394226) was cloned into pcDNA 3.1/Zeo (+) vector (Invitrogen). Transfection into cells was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

RNA interference. We used 5′-AAGUGACAAGUCAAGAGAA-3′ siRNA, the sequence of which is corresponding to nucleotides 175–197 of human dysbindin mRNA. Scrambled siRNA 5′-UUCUCUUGACUUGUCACUU-3′ was used as a negative control. Both sense and antisense strands with two base overhangs were synthesized by NIPPON-EGT (Toyama, Japan) in desalted form. siRNA transfection was performed with Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol.

Immunocytochemistry. Plasmid-transfected cells were fixed with cold 95% ethanol for 7 min at −20 °C and subjected to blocking in 2% BSA/PBS for 10 min. After incubating with an anti-FLAG antibody (1:150, Sigma–Aldrich, St. Louis, USA) overnight, cells were incubated with an Alexa 488 conjugated anti-rabbit secondary antibody (1:500, Invitrogen) for 2 h. siRNA-transfected cells were fixed with cold methanol for 10 min at −20 °C and incubated in PBS containing 5% BSA and 0.3% Triton X-100 for 30 min. The cells were then incubated with an anti-β-tubulin antibody (1:500, Sigma–Aldrich) at 4 °C overnight followed by an Alexa 568 conjugated anti-mouse secondary antibody (1:500, Invitrogen) for 2 h. For detection of actin filament, cells were fixed with 2.5% paraformaldehyde/PBS for 20 min, subjected to permeabilization with 0.1% Triton X-100/PBS for 3 min and then incubated with the Alexa Fluor 568 phalloidin staining solution (5 U/ml, Invitrogen) in PBS containing 1% BSA for 20 min. The coverslips were mounted onto the slides using VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, Peterborough, England). Fluorescence images were acquired using a digital camera DP70 connected with a stereomicroscope (Carl Zeiss, Oberkochen, Germany). Hippocampal cultured neurons at stage 3 were similarly subjected to immunocytochemistry. Fluorescence images were acquired using a confocal laser scanning microscope (LSM-510 UV/META, Carl Zeiss).

Western blot analysis. siRNA-transfected cells were lysed in RIPA buffer containing 1 mM Na_3VO_4, 1 mM NaF and Protease Inhibitor Cocktail (Roche Diagnostics, Basel, Switzerland), incubated for 20 min at 4 °C and centrifuged at 17,000*g* for 20 min at 4 °C. Proteins (3 μg) were separated on SDS–PAGE and electrotransferred onto Immobilon-P Transfer Membranes (MILLIPORE, Billerica, USA). Membranes were incubated in PBS containing 5% skim milk and 0.05% Tween 20 for 1 h and blotted with primary antibodies at 4 °C overnight. An anti-dysbindin antibody (1:1000), anti-phospho-JNK antibody (1:1000, Cell Signaling Technology, Danvers, USA), anti-JNK antibody (1:1000, Cell Signaling Technology) and anti-GAPDH antibody (1:5000, Abcam, Cambridge, USA) were used as primary antibodies. The membranes were incubated with an anti-mouse or anti-rabbit HRP-linked secondary antibody (1:2000, Cell Signaling Technology) for 1 h. Mouse monoclonal anti-dysbindin antibody was produced using the GST fused human dysbindin as antigen. High titer clones to dysbindin were selected by ELISA using the dysbindin protein and the immunoreactivity of the clones were checked by Western blot analysis. For analyses of mice, hemisphere of E16 embryo was homogenized in RIPA buffer and similarly subjected to Western blot analysis.

Results

Portions of dysbindin were localized to the tips of protrusions, and dysbindin knockdown influenced the organization of actin cytoskeleton in SH-SY5Y cells

Dysbindin was previously shown to exist in axon terminals in the hippocampus and to decline in patients with schizophrenia [4,11]. However, dysbindin's function in axon terminals in the hippocampus remains unclear. Thus, to explore the role of dysbindin in neurite formation, we attempted to downregulate it in differentiating SH-SY5Y cells. To clarify how dysbindin operates at the tip of a protrusion, we used an RNA interference method to investigate whether or not alterations in dysbindin expression could influence the morphology of the terminal region of protrusions. Retinoic acid treatment gives rise to the differentiation of SH-SY5Y cells and induces neurite outgrowth [21,22]. Control or dysbindin siRNA-transfected SH-SY5Y cells were incubated with retinoic acid to differentiate and then were analyzed in cytoskeletal organization by visualizing actin filament with rhodamine-phalloidin. Interestingly, the organization of actin cytoskeleton at the tips of neurites of differentiating SH-SY5Y cells was dramatically disrupted by the dysbindin knockdown (Fig. 1A). In addition, immunocytochemical analysis with anti-β-tubulin antibody was performed to compare the lengths of neurites of dysbindin knockdown cells to those of controls. As shown in Fig. 1B, the β-tubulin-positive neurites of dysbindin knockdown cells were apparently shorter than those of the controls. We confirmed that dysbindin with FLAG-tag was expressed in the cell body as well as at the tips of protrusions of SH-SY5Y cells (Fig. 1C). These results suggest that dysbindin knockdown significantly affects the organization of actin cytoskeleton, bringing about the inhibition of neurite outgrowth in differentiating SH-SY5Y cells.

The derangement of cytoskeletal organization was observed in growth cones of hippocampal cultured neurons derived from sdy mice

To confirm the effects of dysbindin knockdown on actin cytoskeleton, we analyzed hippocampal cultured neurons derived from *sdy* mice, which lack dysbindin. The growth cone consists mainly of actin-based structures and can be divided into three distinct regions: the peripheral domain, the central domain, and the transition zone [15]. The peripheral domain has linear actin bundles comprising filopodia and mesh-like gels comprising lamellipodia. The central domain, which is rich in microtubules, has hardly any actin superstructures. In the transition zone located between the peripheral domain and the central domain, transverse bundles of actin filaments are observed. To explore the effects of dysbindin knockdown on actin cytoskeleton in growth cones, hippocampal neurons prepared from E15 mice embryos were cultured, fixed at stage 3, and stained with rhodamine-phalloidin. In neurons derived from wild-type mice, most of the growth cones had a characteristic shape (Fig. 2A). In contrast, the growth cones of neurons derived from *sdy* mice showed significant changes in actin-based

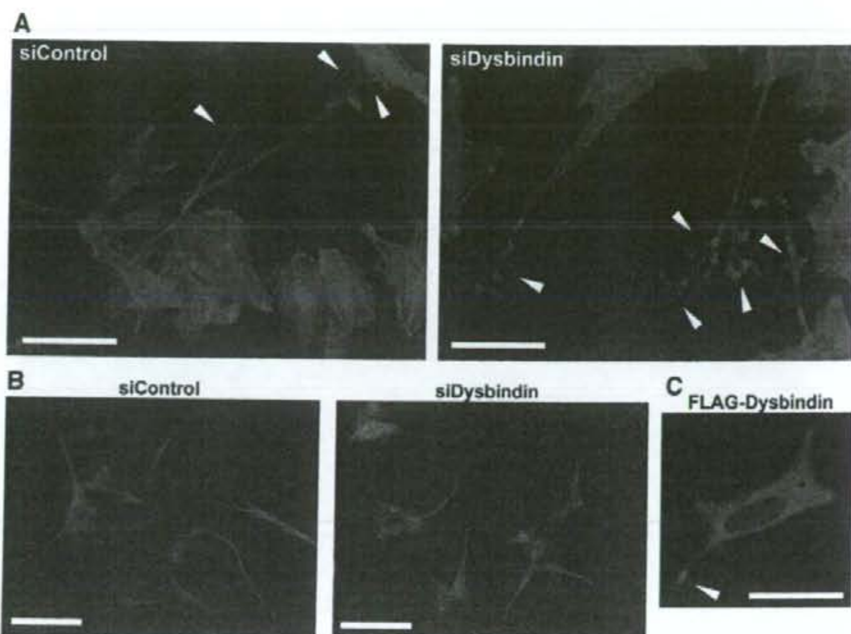

Fig. 1. Dysbindin is involved in neurite morphogenesis. (A) The effects of dysbindin knockdown on actin cytoskeleton. SH-SY5Y cells were transfected with control (siControl) or dysbindin siRNA (siDysbindin), followed by incubation with retinoic acid for 60 h. Actin filament was visualized by rhodamine-phalloidin (red) and DAPI (blue). Bar = 50 μm. (B) The effects of dysbindin knockdown on neurite length. SH-SY5Y cells were transfected with control (siControl) or dysbindin (siDysbindin) siRNA, followed by incubation with retinoic acid for 60 h. The cells were then immunostained using anti-β-tubulin antibody (red) followed by Alexa 568-labeled secondary antibody and DAPI (blue). Bar = 100 μm. (C) Localization of dysbindin in cell body and at the tips of protrusions. SH-SY5Y cells were transfected with FLAG-tagged dysbindin. They were cultured for 24 h and stained with anti-FLAG antibody, followed by Alexa 488-labeled secondary antibody. Bar = 25 μm. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

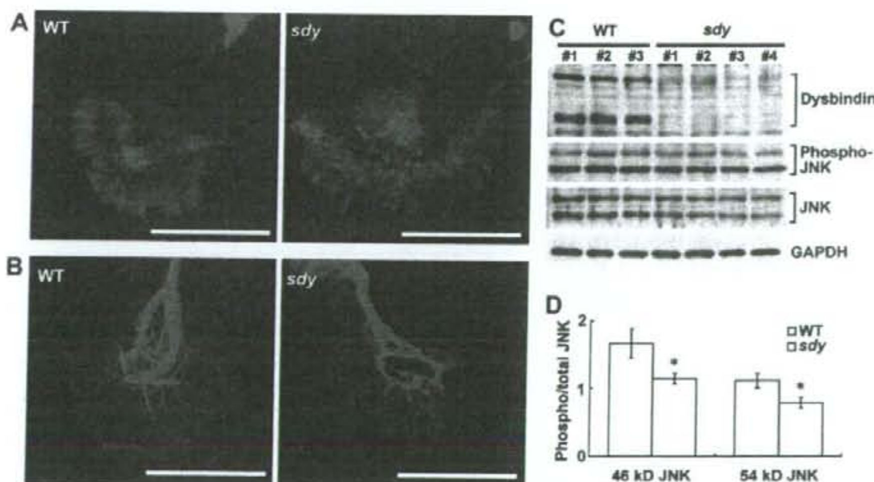

Fig. 2. Dysbindin is involved in the regulation of growth cone morphology. (A) Actin cytoskeleton within the growth cone. Embryonic hippocampi were dissociated from E15 wild-type (WT) or *sdy* mice (*sdy*) and cultured. They were stained with rhodamine-phalloidin to visualize actin filament at stage 3. Bar = 20 μm. (B) Microtubule cytoskeleton within the growth cone. Embryonic hippocampal neurons derived from E15 wild-type (WT) or *sdy* mice (*sdy*) were cultured. The neurons were stained with anti-β-tubulin antibody at stage 3, followed by Alexa 568-labeled secondary antibody. Bar = 20 μm. (C) JNK activity in the brains of wild-type (WT) or *sdy* mice (*sdy*). Lysates homogenized from the hemisphere of E16 embryos were immunoblotted with anti-dysbindin antibody, anti-phosphorylated JNK antibody, anti-JNK antibody, and anti-GAPDH antibody. (D) Quantitated data. Relative ratios of 46 kDa phospho/total JNK and 54 kDa phospho/total JNK in the brains of wild-type (WT) or *sdy* mice (*sdy*) were analyzed using NIH ImageJ software and represented graphically. Statistical comparisons were performed using the unpaired Student's *t*-test. Data represent means ± SD. $^{*}P < 0.05$ versus control.

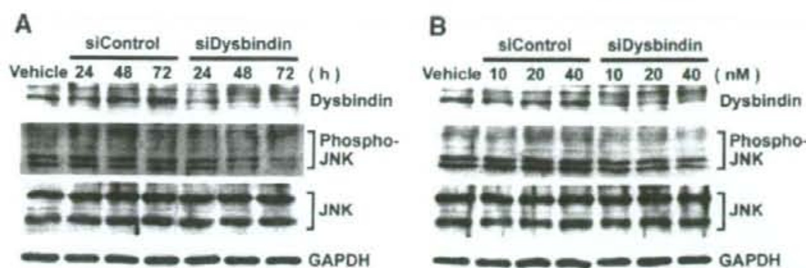

Fig. 3. The JNK phosphorylation level was susceptible to the dysbindin expression level. (A) Time-course dependent effects of dysbindin siRNA on the expression level of dysbindin and phosphorylated JNK. SH-SY5Y cells were transfected with control (siControl) or dysbindin siRNA (siDysbindin), incubated for the indicated times, and subjected to immunoblotting with anti-dysbindin antibody, anti-phosphorylated JNK antibody, anti-JNK antibody, and anti-GAPDH antibody. (B) Dose-dependent effects of dysbindin siRNA on the dysbindin and phosphorylated JNK expression levels. SH-SY5Y cells were transfected with the indicated volumes of control (siControl) or dysbindin siRNA (siDysbindin) for 48 h and harvested. Lysates were immunoblotted with anti-dysbindin antibody, anti-phosphorylated JNK antibody, anti-JNK antibody, and anti-GAPDH antibody.

structures. In those neurons, transverse bundles of actin filament in the transition zone disappeared, and then the central domain became difficult to discern. Additionally, in the peripheral domain, the palm-like shapes consisting of filopodia and lamellipodia were perturbed. On the other hand, microtubules are known to organize into bundles in the neurites, whereas upon entering the central domain of growth cones, they diverge from each other and collaborate with the actin cytoskeleton to contribute growth cone motility and axon elongation [23]. Then, we analyzed microtubule stabilization by staining β-tubulin with a specific antibody. The results revealed that microtubules in the growth cones of neurons derived from *sdy* mice were affected slightly, whereas the controls were unaffected (Fig. 2B). Our findings suggest that hippocampal neurons in *sdy* mice may tend to show the morphological disorder of growth cones.

JNK activity was suggested to be attenuated in the brains of sdy mice embryos

Recent works have shown that activated JNK might play a role in axon formation [20,24]. In hippocampal cultured neurons, JNK has been known to be predominately distributed with cytoskeleton-associated structures such as growth cones [25]. Interestingly, we previously performed pathway analysis with the dysbindin binding proteins, interactions of which were detected by yeast two-hybrid screening, and then found that JNK signaling is a candidate pathway for involvement in dysbindin function (personal communication). Thus, to examine whether or not JNK activity could be altered in the brains of *sdy* mice compared to those of the wild type, a hemisphere from each of seven E16 embryos was homogenized in lysis buffer and subjected to Western blotting analysis. As previously reported [7], we confirmed that the expression of both 50 kDa and 40 kDa dysbindin are abolished in *sdy* mice. Intriguingly, the phosphorylated JNK was lower in the brain lysate derived from *sdy* mice compared to that from the wild type, indicating that dysbindin might regulate the organization of actin cytoskeleton via modulating JNK activity (Fig. 2C and D).

The phosphorylation level of JNK was reduced in proportion with the dysbindin expression level in SH-SY5Y cells

To determine whether or not a reduction in JNK activity was due specifically to the loss of dysbindin, we compared the JNK phosphorylation level with the dysbindin expression level in control or dysbindin siRNA-treated SH-SY5Y cells by Western blot analysis. Though two splice variants of dysbindin exist in DBA/2J mice (Fig. 2C), SH-SY5Y cells predominantly express 50 kDa dysbindin in our experiments. The downregulation of dysbindin was observed in a time-dependent manner in dysbindin siRNA-treated cells, and then phosphorylated JNK was similarly decreased in proportion to the dysbindin expression level (Fig. 3A). In addition, we performed dose–response analysis with each siRNA and found that the expression level of phosphorylated JNK is highly sensitive to that of dysbindin (Fig. 3B). Our data unequivocally demonstrate that there is a significant correlation between dysbindin level and JNK activity.

Discussion

Recent studies have suggested that dysfunction in neurodevelopment and neurotransmission is important for the etiology of schizophrenia [26,27]. For example, DISC1, a candidate gene for susceptibility to schizophrenia, has been known to be part of the NUDEL/LIS1/14-3-3ε complex and to regulate the transport of the protein complex into axons, leading to neuronal migration and axon elongation [28,29]. On the other hand, it has been reported that dysbindin might influence the exocytotic glutamate release and the dopaminergic system via modulation of SNAP25 and synapsin 1 expression [12,13]. Additionally, dysbindin has been suggested to regulate cell surface levels of DRD2 (dopamine D2 receptor) and the strength of the DRD2-mediated G_i signaling pathway [30]. Although a common molecular mechanism under these observations has long been unexplained, morphological abnormalities in developing neurons, which may bring about subsequent dysfunction of synapses, shall be one of the probable causes of susceptibility to schizophrenia.

In this study, we show that dysbindin is required for the normal arrangement of actin cytoskeleton, especially at neurite tips, in differentiating SH-SY5Y cells. Furthermore, we found that the morphological abnormalities are observed in growth cones of cultured hippocampal neurons derived from *sdy* mice, which lack dysbindin. In developing neurons, growth cones are involved in axon elongation and migration [31]. Therefore, the morphological dysfunction of growth cones by a loss of dysbindin may result in an insufficiency of neural circuit formation and synaptogenesis.

Recently, an increasing number of reports have strongly suggested that JNK is relevant to cytoskeletal function [25]. Moreover, it has been demonstrated that phosphorylated JNK was enriched in axons and necessary for proper axon development [20,25]. The JNK family consists of three isoforms: JNK1, JNK2, and JNK3 [32]. Mice devoid of both JNK1 and JNK2 suffer from multiple abnormalities during development of the central nervous system [33]. In addi-

tion, the mutation of JNK3 gene reportedly results in the severe encephalopathy phenotype in children [34]. Interestingly, the present study revealed that the phosphorylation level of JNK is altered by the expression level of dysbindin, raising the possibility that dysbindin functions as a mediator of the JNK signaling pathway, at least at neurite ends, where dysbindin is colocalized with JNK.

Hence, we speculate that a loss of dysbindin results in the failure of normal axon guidance by the disruption of growth cones during the embryonic stage and evokes aberrations in the neurosecretory system in adulthood. However, it remains unclear how dysbindin regulates JNK phosphorylation and how the morphological changes of growth cones in developing neurons contribute to the pathogenic mechanism of schizophrenia. Further analyses are needed to obtain the precise molecular function of dysbindin.

Acknowledgments

This work was in part supported by the 21st Century COE program and by the Osaka Medical Research Foundation for Incurable Diseases. We appreciate Etsuko Moriya and Yoko Ohashi preparing for our experiments.

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American Journal of Medical Genetics Part B: Neuropsychiatric Genetics
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Published Online: 3 Feb 2009
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Research Article

Association studies and gene expression analyses of the DISC1-interacting molecules, pericentrin 2 (*PCNT2*) and DISC1-binding zinc finger protein (*DBZ*), with schizophrenia and with bipolar disorder[†‡]

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‡How to Cite this Article: Anitha A, Nakamura K, Yamada K, Iwayama Y, Toyota T, Takei N, Iwata Y, Suzuki K, Sekine Y, Matsuzaki H, Kawai M, Thanseem I, Miyoshi K, Katayama T, Matsuzaki S, Baba K, Honda A, Hattori T, Shimizu S, Kumamoto N, Kikuchi M, Tohyama M, Yoshikawa T, Mori N. 2009. Association Studies and Gene Expression Analyses of the DISC1-Interacting Molecules, Pericentrin 2 (*PCNT2*) and DISC1-Binding Zinc Finger Protein (*DBZ*), With Schizophrenia and With Bipolar Disorder. Am J Med Genet Part B.
Funded by:
- Ministry of Education, Culture, Sports, Science and Technology of Japan

KEYWORDS

DISC1 •PCNT2 •DBZ •schizophrenia •bipolar disorder

ABSTRACT

Disrupted-in-Schizophrenia 1 (DISC1) and its molecular cascade have been implicated in the pathophysiology of major psychoses. Previously, we identified pericentrin 2 (PCNT2) and DISC1-binding zinc finger protein (DBZ) as binding partners of DISC1; further, we observed elevated expression of *PCNT2* in the postmortem brains and in the lymphocytes of bipolar disorder patients, compared to controls. Here, we examined the association of *PCNT2* with schizophrenia in a case-control study of Japanese cohorts. We also examined the association of *DBZ* with schizophrenia and with bipolar disorder, and compared the mRNA levels of *DBZ* in the postmortem brains of schizophrenia, bipolar and control samples. DNA from 180 schizophrenia patients 201 controls were used for the association study of *PCNT2* and *DBZ* with schizophrenia. Association of *DBZ* with bipolar disorder was examined in DNA from 238 bipolar patients and 240 age- and gender-matched controls. We observed significant

allelic and genotypic associations of the *PCNT2* SNPs, rs2249057, rs2268524, and rs2073380 (Ser/Arg) with schizophrenia; the association of rs2249057 ($P = 0.002$) withstand multiple testing correction. Several two SNP- and three SNP-haplotypes showed significant associations; the associations of haplotypes involving rs2249057 withstand multiple testing correction. No associations were observed for *DBZ* with schizophrenia or with bipolar disorder; further, there was no significant difference between the *DBZ* mRNA levels of control, schizophrenia and bipolar postmortem brains. We suggest a possible role of PCNT2 in the pathogenesis of schizophrenia. Abnormalities of PCNT2, the centrosomal protein essential for microtubule organization, may be suggested to lead to neurodevelopmental abnormalities. © 2009 Wiley-Liss, Inc.

Received: 9 September 2008; Accepted: 9 December 2008

DIGITAL OBJECT IDENTIFIER (DOI)

10.1002/ajmg.b.30926 About DOI

ARTICLE TEXT

INTRODUCTION

Several genetic studies including genome-wide linkage scans and association studies have implicated a potential susceptibility region for psychiatric disorders on chromosome 1q, especially involving the Disrupted-In-Schizophrenia 1 (*DISC1*) gene [Blackwood et al., [2001]; Ekelund et al., [2001]; Hennah et al., [2003]]. *DISC1* has been identified as a disrupted gene by a balanced translocation (1; 11)(q42.1; q14.3) that cosegregated with major psychiatric disorders in a large Scottish kindred [St Clair et al., [1990]; Millar et al., [2000]; Blackwood et al., [2001]; Millar et al., [2001]].

DISC1 variations have been implicated in the positive symptoms of schizophrenia [DeRosse et al., [2007]; Szeszko et al., [2008]], and have been reported to influence the prefrontal function [Prata et al., [2008]; Szeszko et al., [2008]]. *DISC1*-transgenic mice have been found to exhibit brain abnormalities [Kvajo et al., [2008]; Pletnikov et al., [2008]] and behavioral phenotypes [Clapcote et al., [2007]; Hikida et al., [2007]; Li et al., [2007]; Pletnikov et al., [2008]] reminiscent of schizophrenia. Recent studies report the association of *DISC1* polymorphisms with schizophrenia [Hennah et al., [2003]; Kockelkorn et al., [2004]; Callicott et al., [2005]; Sachs et al., [2005]; Zhang et al., [2006]; Chen et al., [2007]; Qu et al., [2007]; Saetre et al., [2008]] and with bipolar disorder [Hodgkinson et al., [2004]; Thomson et al., [2005]].

DISC1 is a multifunctional protein capable of interacting with several cytoskeletal and centrosomal proteins via distinct functional domains [Millar et al., [2003]; Miyoshi et al., [2003]; Morris et al., [2003]; Ozeki et al., [2003]; Brandon et al., [2004]]. Through these interactions, DISC1 functions as a component of the intracellular machinery that integrates multiple functions including intracellular transport, neuronal cell signaling, and neuronal migration and architecture [Hennah et al., [2006]; Ishizuka et al., [2006]; Porteous et al., [2006]]. The impact of DISC1 across several psychiatric diagnostic categories, thus implicates a complex interaction among loci both within the gene itself, and between DISC1 and its multitude of binding partners.

Previously, we reported the fasciculation and elongation protein-zeta 1 [FEZ1; Miyoshi et al., [2003]], pericentrin 2 [PCNT2; Miyoshi et al., [2004]], and DISC1-binding zinc finger protein [DBZ, also known as zinc finger protein 365 (ZNF365); Hattori et al., [2007]] as interacting partners of DISC1. FEZ1, PCNT2, and DBZ bind to overlapping regions of DISC1; in addition, the domain of DISC1 interacting with DBZ is close to the translocation breakpoint in *DISC1* [Hattori et al., [2007]]. *FEZ1* has been found to be associated with schizophrenia in Japanese cohorts [Yamada et al., [2004]]. Lipska et al. [2006] reported reduced mRNA levels of *FEZ1* in the postmortem brain tissues of schizophrenia patients. We observed elevated expression of *PCNT2* in the postmortem brains and in the peripheral blood lymphocytes (PBL) of bipolar disorder patients, compared to healthy controls; however, there was no significant association of *PCNT2* with bipolar disorder [Anitha et al., [2008]]. So far, there are no reports on the association of *DBZ* with any psychiatric disorders.

Here, we examined the genetic association of *PCNT2* with schizophrenia in a case-control study of Japanese cohorts. We also examined the genetic association of *DBZ* with schizophrenia and with bipolar disorder. In addition, we compared the mRNA levels of *DBZ* in the postmortem prefrontal cortices of schizophrenia, bipolar disorder and control groups.

MATERIALS AND METHODS

Association Study

Subjects

This study was approved by the Ethics Committee of Hamamatsu University School of Medicine; patient confidentiality was maintained at all times, and written informed consent was obtained from all the participants. For the association study of *PCNT2* and *DBZ* with schizophrenia, we collected blood samples from 180 schizophrenia patients [age 53.58 7 12 years (mean 7 SD); male/female 99:81] and 201 healthy control subjects (age 40.54 7 13 years; male/female 98:103); there was no significant difference in sex distribution between the case and control groups ($\chi^2 = 1.48$; df = 1; $P = 0.223$). We examined the association of *DBZ* with bipolar disorder, in blood samples collected from 238 bipolar disorder patients (age 51.28 7 13.18 years; male/female 131:107) and 240 age- and gender-matched healthy controls (age 51.49 7 10.73 years, male/female 120:120); the controls samples were independent of the samples used in the association study of *PCNT2* and *DBZ* with schizophrenia. All the subjects were recruited from a geographical area located in central Japan. Best-estimate lifetime diagnoses of patients were made by direct interview with experienced psychiatrists, according to DSM-IV criteria [American Psychiatric Association, [1994]]. Control subjects were recruited from hospital staff and company employees documented to be free from any psychiatric problems.

Genomic DNA was extracted from whole blood using QIAamp DNA blood kit (QIAGEN, Maryland, MD).

Marker selection

The genomic structures of *PCNT2* (chromosomal location 21q22.3) and *DBZ* (10q21.2) are based on the UCSC March 2006 draft assembly of human genome (http://www.genome.ucsc.edu). SNPs for association study were selected using the Applied Biosystems (ABI; Foster City, CA) software SNPbrowser 1.0.19; SNPs reported in databases like National Centre for Biotechnology information (NCBI dbSNP: http://www.ncbi.nlm.nih.gov/SNP) and Japanese Single Nucleotide Polymorphisms (JSNP: http://snp.ims.u-tokyo.ac.jp) were also referred to. On the basis of their genomic locations and minor allele frequencies (MAF > 0.1) in Japanese population, 19 SNPs of *PCNT2* (Fig. 1) and 10 SNPs of *DBZ* (Fig. 2), were chosen for the association study, aiming at an average spacing of one common SNP at every 3-5 kb.

Figure 1. Genomic structure of *PCNT2* and locations of SNPs. Exons are indicated by boxes, with translated regions in closed boxes and untranslated regions in open boxes; SNP positions are denoted by arrows.
[Normal View 5K | Magnified View 11K]

Figure 2. Genomic structure of *DBZ* and locations of SNPs. Exons are indicated by boxes, with translated regions in closed boxes and untranslated regions in open boxes; SNP positions are denoted by arrows.
[Normal View 3K | Magnified View 6K]

Genotyping

Assay-on-Demand^{TM} or Assay-by-Design SNP genotyping products (ABI) were used to score SNPs, based on the TaqMan assay method [Ranade et al., [2001]]. Genotypes were determined in ABI PRISM 7900 Sequence Detection System (SDS), and analyzed using SDS v2.0 (ABI).

Statistical analysis

All the genotyping results were tested for Hardy-Weinberg Equilibrium (HWE). The statistical significance of variations in allelic and genotypic distributions between the schizophrenia and control groups was evaluated using Fisher's exact test. Haplotype associations were examined using the COCAPHASE program of UNPHASED v2.403 [Dudbridge, [2003]; http://www.litbio.org]. Expectation maximization (EM) algorithm was used to resolve uncertain haplotypes, infer missing genotypes and provide maximum-likelihood estimation of frequencies. LD parameters were estimated using the ldmax option of GOLD v1.1.0 [Excoffier and Slatkin, [1995]; Abecasis and Cookson, [2000]; http://www.well.ox.ac.uk/asthma/GOLD/]. LD strength was estimated in terms of the squared correlation coefficient [r^2; Devlin and Risch, [1995]].

Gene Expression Analysis

Brain RNA

RNA from dorsolateral prefrontal cortex (DLPFC; Brodmann's area 46) was donated by The Stanley Medical Research Institute [SMRI; http://www.stanleyresearch.org/programs/brain_collection.asp; Torrey et al., [2000]]. RNA from 31 schizophrenia patients, 33 bipolar disorder patients, and 32 control subjects were used in the study; the demographic details of each group are shown in Table I. Since the RNA samples were coded, the diagnoses of the subjects were masked, while the assays were performed.

Table I. Demographic Characteristics of Control, Schizophrenia and Bipolar Disorder Brain Samples

Variables	Control (N = 32)	Schizophrenia (N = 31)	Bipolar disorder (N = 33)	*P*-value
Age (years) (mean 7 SD)[a]	43.5 7 7.39	42.64 7 8.90	45.41 7 10.70	0.457
Postmortem interval (hr)[a]	29.87 7 13.30	31.23 7 16.50	36.97 7 17.70	0.164
Brain pH[a]	6.63 7 0.25	6.47 7 0.24	6.42 7 0.30	***0.007***
Male/female[b]	23:9	23:8	16:7	***0.039***
Lifetime dose of antipsychotics[cd]	-	89,360 7 105,375	10,339 7 23,181	***<0.001***

[a] One-way ANOVA.
[b] χ^2 test.
[c] Fluphenazine equivalents.
[d] *t*-test.
Bold values indicate significant *P*-values.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Real-time qRT-PCR analysis was performed using the ABI PRISM 7900 SDS. TaqMan primer/probes for *DBZ* and for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), which served as the endogenous reference, were purchased (Assay-on-Demand) from ABI; TaqMan assays were performed in duplicate according to the manufacturer's protocol. A comparative threshold cycle (C_T) method validation experiment was done to check whether the efficiencies of target and reference amplifications were approximately equal (the slope of the log input amount vs. $\Delta C_T < 0.1$). One sample was randomly chosen as the calibrator, and was amplified in each plate, to correct for experimental differences among consecutive PCR runs. The amounts of *DBZ* mRNAs

were normalized to the endogenous reference, and expressed relative to the calibrator as (comparative C_T method).

Statistical analysis

Statistical calculations were performed using the SPSS statistical package, version 11.0.1 (SPSS Co. Ltd., Tokyo, Japan). One-way analysis of variance (ANOVA) was used to check the variability in the distribution of demographic variables and the variability in *DBZ* expression across groups. Any effect of various demographic or clinical variables on *DBZ* expression was examined by Pearson's correlation coefficient.

RESULTS

Association Study of PCNT2 With Schizophrenia

The genotypic distributions of all the SNPs were found to be in HWE in the control group; however, in the schizophrenia group, SNP04 showed a deviation from HWE ($P = 0.015$). The allelic and genotypic frequencies of the 19 SNPs of *PCNT2* in the schizophrenia and control groups are summarized in Table II. SNP04 ($P = 0.002$; OR = 1.6), SNP14 ($P = 0.016$; OR = 1.43), and SNP19 ($P = 0.009$; OR = 1.47) showed significant allelic associations with schizophrenia; the association of SNP04 withstands multiple testing correction. The frequencies of C allele of SNP04 (rs2249057; Exon 10; Ser/Ser), C allele of SNP 14 (rs2268524; Intron 31) and A allele of SNP19 (rs2073380; Exon 45; Ser/Arg) were higher in the schizophrenia group compared to controls. Further, SNP04 ($P = 0.003$), SNP14 ($P = 0.045$), and SNP19 ($P = 0.025$) showed significant variations in the distributions of genotypic frequencies between schizophrenia and control groups.

Table II. Allelic and Genotypic Distributions of *PCNT2* SNPs in Schizophrenia and Control Groups

Marker	SNP	Samples	Allele[a]		*P*-value[b]	Genotype[a]			*P*-value[b]
SNP01	rs2839212		C	T		C/C	C/T	T/T	
	Intron 2	SC (180)	261 (0.73)	99 (0.27)	0.111	96 (0.53)	69 (0.38)	15 (0.09)	0.251
		CT (201)	312 (0.78)	90 (0.22)		121 (0.60)	70 (0.35)	10 (0.05)	
SNP02	rs2839215		G	A		G/G	G/A	A/A	
	Intron 3	SC (180)	260 (0.72)	100 (0.28)	0.156	94 (0.52)	72 (0.40)	14 (0.08)	0.327
		CT (201)	309 (0.77)	93 (0.23)		120 (0.60)	69 (0.34)	12 (0.06)	
SNP03	rs9981892		G	A		G/G	G/A	A/A	
	Intron 5	SC (178)	254 (0.71)	102 (0.29)	0.135	90 (0.51)	74 (0.42)	14 (0.07)	0.308
		CT (200)	305 (0.76)	95 (0.24)		116 (0.58)	73 (0.37)	11 (0.05)	
SNP04	rs2249057		C	A		C/C	C/A	A/A	
	Exon 10 (silent)	SC (180)	230 (0.64)	130 (0.36)	***0.002***	81 (0.45)	68 (0.38)	31 (0.17)	***0.003***
		CT (201)	211 (0.52)	191 (0.48)		56 (0.28)	99 (0.49)	46 (0.23)	
SNP05	rs2839222		A	G		A/A	A/G	G/G	
	Intron 12	SC (178)	258 (0.72)	98 (0.28)	0.075	93 (0.52)	72 (0.40)	13 (0.08)	0.18
		CT (200)	313 (0.78)	87 (0.22)		122 (0.61)	69 (0.35)	9 (0.04)	
SNP06	rs2839224		G	A		G/G	G/A	A/A	
	Intron 13	SC (180)	260 (0.72)	100 (0.28)	0.052	94 (0.52)	72 (0.40)	14 (0.08)	0.138
		CT (200)	314 (0.79)	86 (0.21)		123 (0.62)	68 (0.34)	9 (0.04)	
SNP07	rs6518289		C	T		C/C	C/T	T/T	
	Exon 15 (missense)	SC (180)	258 (0.72)	102 (0.28)	0.114	94 (0.52)	70 (0.39)	16 (0.09)	0.227
		CT (201)	309 (0.77)	93 (0.23)		118 (0.59)	73 (0.36)	10 (0.05)	
SNP08	rs2073378		C	G		C/C	C/G	G/G	
	Intron 16	SC (176)	258 (0.73)	94 (0.27)	0.175	94 (0.53)	70 (0.40)	12 (0.07)	0.379
		CT (201)	312	90		120	72	9 (0.04)	

		(0.78)	(0.22)		(0.60)	(0.36)		
SNP09 rs2268522		G	A		G/G	G/A	A/A	
Intron 21	SC (180)	245 (0.68)	115 (0.32)	0.204	83 (0.46)	79 (0.44)	18 (0.10)	0.57
	CT (201)	291 (0.72)	111 (0.28)		103 (0.51)	82 (0.41)	16 (0.08)	
SNP10 rs762250		G	C		G/G	G/C	C/C	
Intron 21	SC (180)	245 (0.68)	115 (0.32)	0.204	83 (0.46)	79 (0.44)	18 (0.10)	0.417
	CT (201)	291 (0.72)	111 (0.28)		106 (0.53)	79 (0.39)	16 (0.08)	
SNP11 rs2186350		A	G		A/A	A/G	G/G	
Intron 21	SC (179)	243 (0.68)	115 (0.32)	0.202	82 (0.46)	79 (0.44)	18 (0.10)	0.392
	CT (199)	288 (0.72)	110 (0.28)		105 (0.53)	78 (0.39)	16 (0.08)	
SNP12 rs6518291		A	G		A/A	A/G	G/G	
Exon 26 (missense)	SC (180)	262 (0.73)	98 (0.27)	0.051	95 (0.53)	72 (0.40)	13 (0.07)	0.149
	CT (201)	317 (0.79)	85 (0.21)		125 (0.62)	67 (0.33)	9 (0.05)	
SNP13 rs1543756		G	A		G/G	G/A	A/A	
Intron 27	SC (180)	262 (0.73)	98 (0.27)	0.051	95 (0.53)	72 (0.40)	13 (0.07)	0.149
	CT (201)	317 (0.79)	85 (0.21)		125 (0.62)	67 (0.33)	9 (0.05)	
SNP14 rs2268524		C	T		C/C	C/T	T/T	
Intron 31	SC (178)	211 (0.59)	145 (0.41)	***0.016***	63 (0.35)	85 (0.48)	30 (0.17)	***0.045***
	CT (201)	203 (0.50)	199 (0.50)		49 (0.24)	105 (0.52)	47 (0.24)	
SNP15 rs2839251		C	T		C/C	C/T	T/T	
Intron 31	SC (179)	260 (0.73)	98 (0.27)	0.211	94 (0.53)	72 (0.40)	13 (0.07)	0.434
	CT (201)	308 (0.77)	94 (0.23)		117 (0.58)	74 (0.37)	10 (0.05)	
SNP16 rs2070426		C	G		C/C	C/G	G/G	
Exon 37 (missense)	SC (179)	243 (0.68)	115 (0.32)	0.176	82 (0.46)	79 (0.44)	18 (0.10)	0.364
	CT (200)	290 (0.73)	110 (0.27)		105 (0.53)	80 (0.40)	15 (0.07)	
SNP17 rs2073376		G	A		G/G	G/A	A/A	
Exon 38 (missense)	SC (179)	243 (0.68)	115 (0.32)	0.176	82 (0.46)	79 (0.44)	18 (0.10)	0.364
	CT (200)	290 (0.73)	110 (0.27)		105 (0.53)	80 (0.40)	15 (0.07)	
SNP18 rs2051190		T	C		T/T	T/C	C/C	
Intron 41	SC (180)	245 (0.68)	115 (0.32)	0.177	83 (0.46)	79 (0.44)	18 (0.10)	0.374
	CT (201)	292 (0.73)	110 (0.27)		106 (0.53)	80 (0.40)	15 (0.07)	
SNP19 rs2073380		A	C		A/A	A/C	C/C	
Exon 45 (missense)	SC (180)	216 (0.60)	144 (0.40)	***0.009***	66 (0.37)	84 (0.47)	30 (0.16)	***0.025***
	CT (200)	202 (0.50)	198 (0.50)		49 (0.25)	104 (0.52)	47 (0.23)	

SC, schizophrenia; CT, control; the number of genotyped individuals are given in parentheses.

[a] Number followed by frequency in parentheses.

[b] Fisher's Exact test; significant *P*-values are indicated in bold italics.

The *P*-values of haplotypic distributions between schizophrenia and control groups, involving groups of two SNPs (with one SNP overlap) and groups of three SNPs (with two SNP overlap) are given in Table III. Several two-SNP haplotypes showed significant associations; among these, the association of SNP03-SNP04 ($P = 0.001$) reached Bonferroni-corrected significance [0.0027 (α /number of comparisons = 0.05:18)]. Several three-SNP haplotypes also showed significant associations, among which, the

association of SNP03-SNP04-SNP05 (P = 0.002) reached Bonferroni-corrected significance [0.0029 (α/number of comparisons = 0.05:17)].

Table III. Two- and Three-SNP Haplotype Analysis of *PCNT2*

Two-SNP	*P*-value[a]	Three-SNP	*P*-value[a]
1-2	0.182	1-2-3	0.057
2-3	***0.049***	2-3-4	***0.019***
3-4	***0.001***	3-4-5	***0.002***
4-5	***0.006***	4-5-6	***0.004***
5-6	***0.043***	5-6-7	0.086
6-7	0.091	6-7-8	0.297
7-8	0.199	7-8-9	0.129
8-9	0.066	8-9-10	***0.044***
9-10	0.241	9-10-11	0.225
10-11	0.178	10-11-12	***0.008***
11-12	***0.008***	11-12-13	0.055
12-13	0.055	12-13-14	***0.047***
13-14	***0.039***	13-14-15	***0.004***
14-15	0.061	14-15-16	0.246
15-16	***0.038***	15-16-17	***0.03***
16-17	0.162	16-17-18	0.162
17-18	0.164	17-18-19	***0.017***
18-19	***0.023***		

[a] Significant *P*-values are indicated in bold italics.

A graphic representation of the LD strength (r^2 values) between markers in the schizophrenia group is shown in Figure S1; strong LD (r^2 = 0.920) was observed between SNPs 14 and 19, which showed associations with schizophrenia. The LD pattern was found to be similar in the schizophrenia and control (data not shown) groups.

Association Study of DBZ With Schizophrenia

The allelic and genotypic frequencies of the 10 SNPs of *DBZ* are given in Table IV. Genotypic distributions of all the SNPs were found to be in HWE, in the schizophrenia and control groups. None of the SNPs showed any significant associations with schizophrenia. No significant haplotype associations were observed in the two-SNP or three-SNP haplotype analyses.

Table IV. Allelic and Genotypic Distributions of *DBZ* SNPs in Schizophrenia and Control Groups

Marker	SNP	Samples	Allele[a]		*P*-value[b]	Genotype[a]			*P*-value[b]
SNP01	rs10995132		G	A		G/G	G/A	A/A	
	5′	SC (179)	311 (0.87)	47 (0.13)	0.660	135 (0.75)	41 (0.23)	3 (0.02)	0.794
		CT (199)	345 (0.86)	47 (0.14)		148 (0.74)	49 (0.24)	2 (0.02)	
SNP02	rs3844347		A	G		A/A	A/G	G/G	
	5′	SC (180)	288 (0.80)	72 (0.20)	0.426	116 (0.65)	56 (0.31)	8 (0.04)	0.541
		CT (201)	311 (0.77)	91 (0.23)		119 (0.59)	73 (0.36)	9 (0.05)	
SNP03	rs3781213		G	C		G/G	G/C	C/C	
	Intron 1	SC (180)	287 (0.79)	73 (0.21)	0.428	115 (0.64)	57 (0.31)	8 (0.05)	0.561
		CT (201)	310 (0.77)	92 (0.23)		118 (0.59)	74 (0.36)	9 (0.05)	
SNP04	rs1873687		T	C		T/T	T/C	C/C	
	Intron 2	SC (180)	285 (0.79)	75 (0.21)	0.659	114 (0.63)	57 (0.32)	9 (0.05)	0.560
		CT (200)	311 (0.78)	89 (0.22)		119 (0.60)	73 (0.36)	8 (0.04)	

SNP05	rs10761627		T	C		T/T	T/C	C/C	
	Intron 2	SC (180)	285 (0.79)	75 (0.21)	0.539	114 (0.63)	57 (0.32)	9 (0.05)	0.595
		CT (200)	309 (0.77)	91 (0.23)		118 (0.59)	73 (0.37)	9 (0.04)	
SNP06	rs7075904		C	G		C/C	C/G	G/G	
	Intron 2	SC (180)	309 (0.86)	51 (0.14)	0.917	133 (0.74)	43 (0.24)	4 (0.02)	1.000
		CT (200)	345 (0.86)	55 (0.14)		149 (0.74)	47 (0.24)	4 (0.02)	
SNP07	rs7070152		A	G		A/A	A/G	G/G	
	Intron 3	SC (180)	286 (0.79)	74 (0.21)	0.857	116 (0.64)	54 (0.30)	10 (0.06)	0.544
		CT (201)	322 (0.80)	80 (0.20)		128 (0.64)	66 (0.33)	7 (0.03)	
SNP08	rs3758490		T	G		T/T	T/G	G/G	
	Exon 5 (Ser/Ala)	SC (176)	221 (0.63)	131 (0.37)	0.063	66 (0.37)	89 (0.51)	21 (0.12)	0.143
		CT (199)	276 (0.69)	122 (0.31)		93 (0.47)	90 (0.45)	16 (0.08)	
SNP09	rs4746698		A	G		A/A	A/G	G/G	
	3′	SC (179)	246 (0.69)	112 (0.31)	0.641	87 (0.49)	72 (0.40)	20 (0.11)	0.301
		CT (200)	268 (0.67)	132 (0.33)		86 (0.43)	96 (0.48)	18 (0.09)	
SNP10	rs2893900		C	T		C/C	C/T	T/T	
	3′	SC (179)	337 (0.94)	21 (0.06)	0.207	159 (0.89)	19 (0.10)	1 (0.01)	0.249
		CT (201)	368 (0.91)	34 (0.09)		168 (0.83)	32 (0.16)	1 (0.01)	

SC, schizophrenia; CT, control; the number of genotyped individuals are given in parentheses.
[a] Number followed by frequency in parentheses.
[b] Fisher's Exact test.

Association Study of DBZ With Bipolar Disorder

The allelic and genotypic frequencies of the 10 SNPs of *DBZ* are shown in Table V. The genotypic distributions of all the SNPs were found to be in HWE in the control group; however, SNP09 (rs4746698) showed a marginal deviation from HWE, in the bipolar group ($P = 0.033$). None of the SNPs showed any significant associations with bipolar disorder. No significant haplotype associations were observed in the two-SNP or three-SNP haplotype analyses.

Table V. Allelic and Genotypic Distributions of *DBZ* SNPs in Bipolar Disorder and Control Groups

Marker	SNP	Samples	Allele[a]		*P*-value[b]	Genotype[a]			*P*-value[b]
SNP01	rs10995132		G	A		G/G	G/A	A/A	
	5′	BD (237)	407 (0.86)	67 (0.14)	0.783	174 (0.73)	59 (0.25)	4 (0.02)	0.801
		CT (240)	409 (0.85)	71 (0.15)		172 (0.72)	65 (0.27)	3 (0.01)	
SNP02	rs3844347		A	G		A/A	A/G	G/G	
	5′	BD (238)	372 (0.78)	104 (0.22)	0.489	146 (0.61)	80 (0.34)	12 (0.05)	0.565
		CT (240)	366 (0.76)	114 (0.24)		137 (0.57)	92 (0.38)	11 (0.05)	
SNP03	rs3781213		G	C		G/G	G/C	C/C	
	Intron 1	BD (238)	367 (0.77)	109 (0.23)	0.818	143 (0.60)	81 (0.34)	14 (0.06)	0.443
		CT (239)	365 (0.76)	113 (0.24)		136 (0.57)	93 (0.39)	10 (0.04)	
SNP04	rs1873687		T	C		T/T	T/C	C/C	
	Intron 2	BD (236)	368 (0.78)	104 (0.22)	0.489	145 (0.62)	78 (0.33)	13 (0.05)	0.419