クリジン(Phencyclidine: PCP)を急性投与したマウスを用いた。PCP は、ヒトにおいて統合失調症の主要な３つの症状全て、すなわち陽性症状と陰性症状、認知機能障害と類似した行動を引き起こすことが知られており(Javitt and Zukin, *Am J Psychiatry*, 1991)、またサルやマウスなどの実験動物においても、認知機能障害を含む統合失調症様の症状が再現されることが知られている。実験を始めるにあたりまず、マウス新生仔内側前頭皮質へ、多くの大脳皮質 GABA 細胞を産生することが知られているマウス胎仔内側基底核原基(Medial ganglionic eminence: MGE)細胞を移植し、移植後 3 ヶ月目に GABA 細胞が生着していることを確かめた(図 1)。今後、次に移植細胞の、認知機能障害に対する予防効果を検証するために、移植後 1.5 ヶ月目に PCP を急性投与し、認知機能を計測するテストを行う。

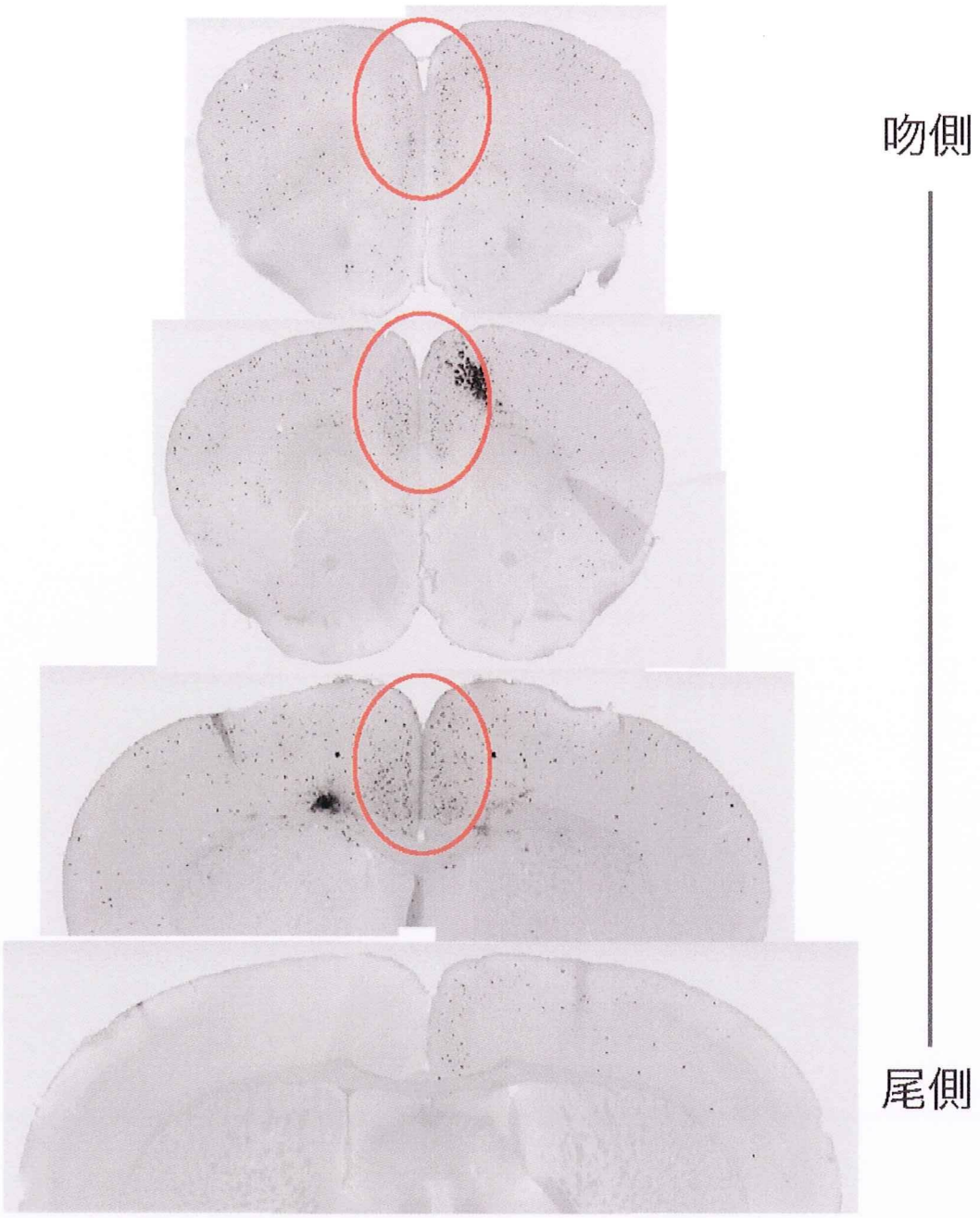

図1．マウス新生仔内側前頭皮質への移植から3ヶ月後の移植細胞(黒点)の分布(冠状断)

内側前頭皮質(赤○領域)に多くの細胞が生着している

D. 考察

本研究は、統合失調症の病態理解に役立てる事を目的として、統合失調症候補遺伝子 *DISC1* の機能を解析した。子宮内胎児電気穿孔法を用いた前頭前野への部位特異的な遺伝子導入法を確立し、それと siRNA 技術を組み合わせることで、簡単に部位特異的な遺伝子機能調節が可能になった。これまでのノックアウトマウスでの解析に比べてはるかに容易に遺伝子の機能調節が可能になったことで、特定の脳部位での遺伝子機能を解析するうえで重要な研究手法が得られた。

さらに本研究では、特定の脳部位での遺伝子機能が成熟後の行動に与える影響にまで踏み込むことができた。本研究で用いた子宮内胎児電気穿孔法は所属研究室で開発され、現在特許出願中である。この手法は子宮内のマウス胎仔脳に任意の遺伝子を導入することができ、特にその高い解析効率（簡便かつ迅速）ですぐれている。国内外で、トランスジェニックマウスやノックアウトマウスの作製が行われると考えられるが、それらとは相補的な知見が得られたと考えられる。この方法は、また、それらの実験系よりも、はるかに短期間のうちに多くの種類の解析を行うことができ、その上、全身でノックダウンした場合に致死となってしまうような遺伝子でも、時期及び部位特異的に遺伝子操作が行えることで、目的の時期・部位での機能の解析が容易である利点がある。また、それらの遺伝子改変動物が作成されたとしても、本研究で用いた子宮内マウス胎児脳電気穿孔法を用いた手法においては、1）周囲は正常な環境下で、遺伝子導入がなされた細胞においてのみ遺伝子のノックダウンが起こるため、cell-intrinsic な機能が明らかになりやすい、2）発生の途中から、急激にノックダウンが起こるため、他の遺伝子発現の調節等による形質の redundancy が起こりにくい、などの特徴がある。これらの特徴により、本研究の手法を応用していくことで、遺伝子機能がより明確になる可能性が期待される。

統合失調症の脳の病理所見として、これまでに死後脳を用いた研究から、シナプスの減少、海馬や辺縁系での組織異常（Arnold SE, Trojanowski JQ. Recent advances in defining the neuropathology of schizophrenia. Acta Neuropathol (Berl). 1996 Sep;92(3):217-31）、大脳皮質での GABA の減少（Lewis DA *et al.* Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci. 2005 Apr;6(4):312-24）など多彩な所

見が報告されている。今回、我々は子宮内胎児電気穿孔法を用いた前頭前野への部位特異的な遺伝子導入法を確立したが、その結果、前頭前野における parvalbumin 陽性の GABA 作動性抑制性神経細胞の減少が観察された。統合失調症の病理仮説として、長年ドーパミン仮説が有力であったが、死後脳の病理所見からは、前頭葉や海馬の抑制性 GABA 作動性神経細胞の機能低下が指摘されている（Lewis DA, *et al.*, Nat Rev Neurosci. 2005 Apr;6(4):312-24.)。

加えて、本研究では GABA 作動性抑制性神経細胞特異的な遺伝子導入法を開発し、GABA 作動性抑制性神経細胞の発生段階においても DISC1 の機能が重要であることを明らかにした。逆に DISC1 の機能阻害が起こると、GABA 作動性抑制性神経細胞にも機能異常が起こることが十分予想される結果となった。GABA を介した興奮性神経細胞の抑制の不全は統合失調症における認知機能低下の要因とも考えられている（Daskalakis ZJ, *et al.*, Brain Res Rev. 2007 Dec;56(2):427-42.)ため、本研究をさらに発展させることにより、統合失調症における認知機能低下の病態理解に有用であることが期待される。また、研究分担者である田中が示したように、GABA 作動性抑制性神経細胞は生後も高い移動能を保ち，細胞補充療法のよいツールとして注目されているため，今後これらの細胞における病態が明らかになれば，たとえば成体において抑制性神経細胞の発生分化や移動・配置を調節するような化合物が治療薬となりうる可能性がある。

精神疾患、中でも統合失調症は、いまなお慢性の経過をたどることが多く、社会的な側面からも患者・家族の負担は大きい。このためその病因、病態の把握は急務である。統合失調症の脳の病理組織では、各脳領域に微細な組織構築の乱れがあると考えられている。これまで、これらの形態・組織構築の異常がどのような分子異常に基づいて起こるのか不明であったが、近年の家系を用いた連鎖解析から、*DISC1* をはじめとする統合失調症の有力な候補遺伝子が見いだされてきている。一般的には、大部分の精神疾患は単一の遺伝子によって引き起こされるのではなく、多因子性の複雑遺伝疾患であると予想される。しかし、アルツハイマー病などの神経変性疾患に関する研究の進展もまれな家族例からの遺伝子の発見を契機としており、こうした候補遺伝子に立脚した病態研究から多くの知見が得られると期待され、*DISC1* の解析を手がかりとして、統合失調症の生物学的研究

および病態理解を進めていきたいと考えている。本研究の結果、DISC1の機能異常によって生じる分子病態・神経病態の解明については、その機能異常が与える様々な影響が明らかになった。また発生過程における分子的異常が、成熟後のマウスに統合失調様の行動異常を引き起こすことが明らかになり、これまで不明であった統合失調症発症の分子的実体を提示することができた。

これは、方法論的な意義が大きいのみならず、病態理解の上で重要であると考えられる。本研究で標的とした前頭前野は、統合失調症における認知機能障害の重要な責任部位であると考えられる。この認知機能障害は日常生活機能の障害につながり、社会復帰から自殺企図までを含めた社会予後全般と深く関わるとされる。このため、この認知機能障害の病態を理解し、それに対する治療戦略を得ることは、社会的入院を減らし、自殺件数を減少させることにもつながるため、行政的な意義も大きいと考えられる。

さらに本研究ではGABA作動性神経細胞の移植を用いた治療戦略の構築を行った。細胞移植による神経疾患の治療はこれまで特にパーキンソン病や脊髄損傷において採用され一定の成果を上げているが、統合失調症に対する予防および治療を目的とした細胞移植の研究は実験モデル動物での基礎研究も含め全く報告がない。本研究がおそらく統合失調症に対する予防および治療戦略として最初の事例であり、極めて独自性の高いアプローチを考案できたと考えている。

本研究の提示した予防・再発予防策である細胞補充療法は、本研究のような動物を対象とした基礎的な研究でしか検証のできない全く新しい手法である。それをそのまま実際の治療での実行に移す可能性は低いが、「(特定の性質の)神経細胞を増やすこと(あるいは、そのような刺激を加えること)が統合失調症予防(あるいは再発予防)・治療に有効か否か」という命題に対して一つの答えを与えるものである。抗うつ薬が脳内の神経細胞新生を増加させるという知見が集まりつつあるが、「どのような神経細胞をどこで増加させる必要があるか」など、より焦点をしぼった研究開発に結びつく可能性がある点で意義があると考えている。

また、この細胞補充療法は、研究開発の方向性に新たなヒントを与える上で重要である。現在手詰まりとなっている新規薬剤開発の新たな方向性を見いだすうえでも、あるいは全く新しい治療方法を開発するうえでも、その一助となることが期待される。現時

点では想像しにくいが、人工多能性幹細胞（iPS 細胞）に関する研究と結びついて画期的な治療法を生みだす可能性も否定できない。その場合、我が国で発見され、世界に先駆けてその実用化を進めることが国家戦略となっている、人工多能性幹細胞（iPS 細胞）作成技術を精神神経科領域で応用する上での道標となる可能性がある。

E. 結論

本研究の結果、発生段階における分子的異常が神経細胞の発生・発達に障害を及ぼし、成熟後の統合失調症発症を準備する可能性が示唆された。また本研究で開発されたマウスモデルは、統合失調症における認知機能低下の病態理解および治療戦略の構築に有用であることが期待される。

加えて、本研究ではGABA作動性神経細胞(GABA細胞)を前頭葉に移植することで、統合失調症認知機能障害を予防・治療する、新規かつ独自の治療戦略を構築した。

F. 研究発表

発表論文

Minae Niwa, Atsushi Kamiya, Rina Murai, Ken-ichiro Kubo, Aaron J Gruber, Kenji Tomita, Lingling Lu, Shuta Tomisato, Hanna Jaaro-Peled, Saurav Seshadri, Hideki Hiyama, Beverly Huang, Kazuhisa Kohda, Yukihiro Noda, Patricio O'Donnell, Kazunori Nakajima, Akira Sawa, and Toshitaka Nabeshima. Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. ***Neuron*** , 65 (4), 480-489 (2010).

Atsushi Kamiya, Perciliz L. Tan, Ken-ichiro Kubo, Caitlin Engelhard,Koko Ishizuka, Akiharu Kubo, Sachiko Tsukita, Ann E. Pulver, Kazunori Nakajima, Nicola G. Cascella, Nicholas Katsanis, and Akira Sawa. Recruitment of PCM1 to the centrosome by the cooperative action of DISC1 and BBS4: a candidate for psychiatric illness. ***Arch. Gen. Psychiatry***, 65 (9), 996-1006 (2008)

学会発表

Ishizuka K., Kamiya A., Oh E., Robinson J., Mitsuma N., Furukori K., Seshadri S., Huang B., Kubo K., Nakajima K., Katsanis N., Sawa A. "Phosphorylation of DISC1 activates a molecular switch from cell proliferation to neuronal migration in the developing cortex"Society for Neuroscience, Neuroscience 2009 Meeting (39th Annual Meeting), Chicago, U.S.A., 2009.10.17-21

丹羽美苗、神谷篤、村井里菜、久保健一郎、Hanna Jaaro-Peled、陸玲玲、尾崎紀夫、仲嶋一範、野田幸裕、澤明、鍋島俊隆"発生期大脳皮質における Disrupted-in-Schizophrenia-1 の発現異常はドパミン作動性神経系機能異常を伴う精神疾患表現型を発現する（Disturbance of Disrupted-in-Schizophrenia-1 during neurodevelopment develops phenotypes of psychiatric disorders related to dysfunction of the dopaminergic system）"シンポジウム："統合失調症"第 32 回日本神経科学大会、名古屋、2009 年 9 月 16-18 日

Kenji Tomita, Ken-ichiro Kubo, Asuka Uto, Atsushi Kamiya, Akira Sawa, and Kazunori Nakajima"The analysis of the role of DISC1

in the cortical neuronal migration"第38回慶應ニューロサイエンス研究会、東京、2009年3月14日

宇都飛鳥、久保健一郎、金谷繁明、仲嶋一範"大脳皮質抑制性神経細胞におけるDISC1の機能解析"日本解剖学会関東支部第96回学術集会、つくば、2008年11月22日

Atsushi Kamiya, Minae Niwa, Rina Murai, Yukihiro Noda, Ken-ichiro Kubo,Hanna Jaaro-Peled, Saurav Seshadri, Kazunori Nakajima, Toshitaka Nabeshima,and Akira Sawa "Genetic disturbance of cortical development: exploring novel animal models for schizophrenia via in utero gene transfer"Society for Neuroscience, 38th Annual Meeting, 2008.11.15-19, Washington,D.C., U.S.A.

Toshitaka Nabeshima, Minae Niwa, Rina Murai, Yukihiro Noda, Ken-ichiro Kubo, Atsushi Kamiya, Hanna Jaaro-Peled, Kazunori Nakajima, and Akira Sawa "Transient knockdown of DISC1 in the developing cerebral cortex leads to dopaminergic disturbance and schizophrenia-like deficits in young adult mice "Symposium : "Animal models of neuropsychiatric disorders ? from C. elegans to monkey -第51回日本神経化学会大会、2008.9.11-13、富山

Kenji Tomita, Ken-ichiro Kubo, Asuka Uto, Atsushi Kamiya, Akira Sawa, and Kazunori Nakajima "The analysis of the role of DISC1 in the brain layer formation in vivo"第31回日本神経科学大会、東京、2008年7月9-11日

Ken-ichiro Kubo, Atsushi Kamiya, Akira Sawa, and Kazunori Nakajima "The analysis of the role of DISC1 in the neuronal migration of the neocortex in vivo"Society for Neuroscience, 37th Annual Meeting, San Diego, CA, U.S.A., 2007 年 11 月 3-7 日

久保健一郎、神谷篤、澤明、仲嶋一範"新皮質の形成において DISC1 が果たす役割の解析"第 30 回日本神経科学大会・第 50 回日本神経化学会大会・第 17 回日本神経回路学会大会合同大会（Neuro2007）、横浜、2007 年 9 月 10-12 日

久保健一郎、神谷篤、澤明、仲嶋一範"大脳皮質神経細胞移動における DISC1 の役割の解析"第 112 回日本解剖学会全国学術集会、大阪、2007 年 3 月

G．知的財産権の取得状況

特になし

研究成果の刊行に関する一覧表

著者氏名	論文タイトル名	発表誌名
Atsushi Kamiya, Perciliz L. Tan, Ken-ichiro Kubo, Caitlin Engelhard, Koko Ishizuka, Akiharu Kubo, Sachiko Tsukita, Ann E. Pulver, Kazunori Nakajima, Nicola G. Cascella, Nicholas Katsanis, Akira Sawa	Recruitment of PCM1 to the centrosome by the cooperative action of DISC1 and BBS4: a candidate for psychiatric illness	***Arch. Gen. Psychiatry***, 65 (9), 996-1006 (2008)
Minae Niwa, Atsushi Kamiya, Rina Murai, Ken-ichiro Kubo, Aaron J Gruber, Kenji Tomita, Lingling Lu, Shuta Tomisato, Hanna Jaaro-Peled, Saurav Seshadri, Hideki Hiyama, Beverly Huang, Kazuhisa Kohda, Yukihiro Noda, Patricio O'Donnell, Kazunori Nakajima, Akira Sawa, and Toshitaka Nabeshima	Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits.	***Neuron***, 65 (4), 480-489 (2010).

ORIGINAL ARTICLE

Recruitment of PCM1 to the Centrosome by the Cooperative Action of DISC1 and BBS4

A Candidate for Psychiatric Illnesses

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Context: A role for the centrosome has been suggested in the pathology of major mental illnesses, especially schizophrenia (SZ).

Objectives: To show that pericentriolar material 1 protein (PCM1) forms a complex at the centrosome with disrupted-in-schizophrenia 1 (DISC1) and Bardet-Biedl syndrome 4 protein (BBS4), which provides a crucial pathway for cortical development associated with the pathology of SZ. To identify mutations in the *PCM1* gene in an SZ population.

Design: Interaction of DISC1, PCM1, and BBS proteins was assessed by immunofluorescent staining and coimmunoprecipitation. Effects of PCM1, DISC1, and BBS on centrosomal functions and corticogenesis in vivo were tested by RNA interference. The *PCM1* gene was examined by sequencing 39 exons and flanking splice sites.

Setting: Probands and controls were from the collection of one of us (A.E.P.).

Patients: Thirty-two probands with SZ from families that had excess allele sharing among affected individuals at 8p22 and 219 white controls.

Main Outcome Measures: Protein interaction and recruitment at the centrosome in cells; neuronal migration in the cerebral cortex; and variant discovery in *PCM1* in patients with SZ.

Results: PCM1 forms a complex with DISC1 and BBS4 through discrete binding domains in each protein. DISC1 and BBS4 are required for targeting PCM1 and other cargo proteins, such as ninein, to the centrosome in a synergistic manner. In the developing cerebral cortex, suppression of PCM1 leads to neuronal migration defects, which are phenocopied by the suppression of either DISC1 or BBS4 and are exacerbated by the concomitant suppression of both. Furthermore, a nonsense mutation that segregates with SZ spectrum psychosis was found in 1 family.

Conclusions: Our data further support for the role of centrosomal proteins in cortical development and suggest that perturbation of centrosomal function contributes to the development of mental diseases, including SZ.

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RECENT GENETIC STUDIES have suggested that centrosomal dysfunction underlies risks for various neuropsychiatric disorders, because variants in some genes that encode centrosomal proteins have been associated with schizophrenia (SZ) and bipolar disorder (BP).[1-4] These genes include pericentriolar material 1 *(PCM1)* on chromosome 8p22,[2] one of the reproducible linkage loci for SZ and BP,[5-8] and disrupted-in-schizophrenia 1 *(DISC1)*.[3,4] The centrosome plays a role in organizing microtubules, contributing to cell cycle progression, cell polarization, and ciliogenesis.[9-12] Consequently, the centrosome is required for proper neurodevelopment, especially in the cerebral cortex.[13-17]

PCM1 is a component of centriolar satellites and acts as a scaffold to target several proteins to the centrosome in a dynein motor-dependent manner and regulate microtubular dynamics.[18-20] PCM1 also interacts with Bardet-Biedl syndrome 4 protein (BBS4), which is encoded by one of the causative genes for Bardet-Biedl syndrome (BBS), an inherited disorder characterized by renal dysfunction, obesity, polydactyly, and diverse neuropsychiatric symptoms.[21-24] Bardet-Biedl syndrome is genetically heterogeneous, with 12 genes identified to date, but mutations in each of these genes lead to similar pathology in humans, suggesting that BBS proteins function through a common molecular pathway. Consistent with this notion, all BBS proteins investigated to date localize pri-

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marily at the centrosome and the basal body of ciliated cells, where they contribute to the maintenance of microtubular dynamics, as well as intracellular transport and ciliary function.[25-30]

We have reported previously that DISC1, a major susceptibility factor for SZ and BP, plays a crucial role at the centrosome,[31,32] while another group has reported consistently that DISC1 interacts with kendrin, a component of pericentriolar material.[33] Consequently, DISC1 is required for neurite outgrowth and proper development of the cerebral cortex, such as neuronal migration and dendritic arborization.[31] Therefore, we hypothesized that PCM1, DISC1, and the BBS proteins may interact and play a role in the centrosome and that such interactions might be relevant both to the DISC-associated neurodevelopmental functions and to the etiopathology of SZ.

Herein, we provide biological and genetic evidence that PCM1-DISC1-BBS proteins form a centrosomal pathway, potentially associating with major mental illnesses, such as SZ. These proteins form a complex at the centrosome through discrete binding domains. DISC1 and BBS4 act synergistically to recruit PCM1 and associated proteins to the centrosome. Disruption of the PCM1-DISC1-BBS4 pathway leads to profound defects in neuronal migration during cortical development. Finally, we report a pedigree in which a nonsense mutation in the *PCM1* gene segregates with SZ spectrum psychosis.

METHODS

PLASMIDS AND ANTIBODIES

All the deletion DISC1 and PCM1 expression constructs were made by polymerase chain reaction–based mutagenesis protocol.[34] The deletion BBS4 expression constructs were made as described previously.[21] pEGFP-F was purchased from BD Bioscience Clontech (Mountain View, California). Rabbit polyclonal antibodies against PCM1, ninein, BBS1, BBS4, and BBS8 antibody were prepared as described previously.[20,21,25,35] The following antibodies were also used: mouse monoclonal antibodies against β-tubulin and γ-tubulin (Sigma-Aldrich, St Louis, Missouri); mouse monoclonal antibodies against HA-tag and myc-tag (BAbCO, Berkeley, California); rabbit polyclonal antibody against HA-tag (Clontech); rabbit polyclonal antibody against myc-tag (Santa Cruz Biotechnology, Santa Cruz, California); affinity-purified rabbit antiserum against green fluorescent protein (GFP) (Molecular Probes, Eugene, Oregon); and mouse monoclonal antibody against GFP (Nacalai Tesque, Kyoto, Japan). The rabbit polyclonal anti-DISC1 antibody (D27) was a gift from Nicholas J. Brandon, PhD (Wyeth Discovery Neuroscience). Plasmids expressing interfering short hairpin RNA (shRNA)[36] were generated to suppress endogenous DISC1, PCM1, and BBS4 protein expression. Their target sequences were as follows: DISC1 RNA interference (RNAi), 5′-GGCAAACACTGTGAAGTGC-3′; PCM1 RNAi, 5′-TCAGCTTCGTGATTCTCAG-3′; and BBS4 RNAi, 5′-GCAGCTATCAGCTGCCTAA-3′.

A scrambled sequence without homology to any known messenger RNA was used to produce the control RNAi. The efficiency of all shRNAs was tested by the extent of suppression in endogenous target protein in rat PC12 cells by Western blotting.

CELL CULTURE AND TRANSFECTION

HEK293 cells were maintained in Dulbecco's modified Eagle medium with 10% fetal bovine serum and 1% penicillin-streptomycin. PC12 cells were maintained in Dulbecco's modified Eagle medium with 10% fetal bovine serum, 5% horse serum, and 1% penicillin-streptomycin. Transfection of expression constructs or RNAi constructs was carried out with Lipofectamine 2000 (Invitrogen, Carlsbad, California) for PC12 cells and with PolyFect Transfection Reagent (Qiagen, Valencia, California) for HEK293 cells. The molar ratio of pEGFP-F to RNAi plasmid(s) was 1:3 for the transfection. Rodent primary cortical neurons were prepared as described previously.[37]

COIMMUNOPRECIPITATION AND CELL EXTRACTION

Immunoprecipitation

Cells were lysed in a RIPA buffer (50mM TRIS–hydrogen chloride, pH 7.4, 150mM sodium chloride, 5mM magnesium chloride, 5mM dithiothreitol, 1mM phenylmethylsulfonyl fluoride, 1mM ethylene diamine tetraacetic acid, 1% Triton X-100, and protease inhibitor mixture [Roche, Basel, Switzerland]). Precleared supernatants (500 µg) from crude cell lysates centrifuged at 14 000 × *g* for 10 minutes were incubated with primary antibodies (1 µg/mL of rabbit polyclonal antibody against HA-tag or against myc-tag) overnight, which was followed by the addition of TrueBlot anti-Rabbit Ig IP Beads (eBioscience, San Diego, California) (30 µL) or Protein G Plus/Protein A Agarose (Calbiochem, Darmstadt, Germany) (30 µL) for 1 hour. The immunoprecipitates were washed 3 times by a TRIS-buffered saline–based buffer with 0.05% Tween 20 and analyzed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)/Western blotting. In the stringent wash conditions, we added sodium chloride up to the final concentration at 500mM. ProFound Mammalian HA Tag IP/Co-IP Kit (Pierce, Rockford, Illinois) was also used.

Cell Extraction

Cells were sonicated in ice-cold lysis buffer (50mM TRIS–hydrogen chloride, pH 7.4, 150mM sodium chloride, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and a protease inhibitor mixture). Extracted cells were mixed with SDS-PAGE loading buffer after protein concentrations were measured. Each protein sample (10 µg) was analyzed with SDS-PAGE followed by Western blotting.

IMMUNOFLUORESCENT STAINING

Cells were fixed with ice-cold methanol at −20°C 3 days after transfection. After blocking with 1.5% bovine serum albumin and 0.5% normal goat serum in phosphate-buffered saline, cells were treated with primary antibodies (dilution: γ-tubulin, 1:100; DISC1, 1:200; PCM1, 1:500; ninein, 1:500; BBS1, 1:300; BBS4, 1:500; BBS8, 1:500) for 1 hour followed by the reaction with secondary antibodies conjugated to Rhodamine Red-X (dilution, 1:300) and Cy5 (dilution, 1:300) (Jackson ImmunoResearch, West Grove, Pennsylvania) for 1 hour. Hoechst 33258 (Molecular Probes) was used at 1:500 dilution for 3 minutes to visualize nuclei. Confocal microscopy (LSM 510 Meta; Zeiss, Grottingem, Germany) was used for epifluorescent image collection. To obtain clearer images of cell morphology under methanol fixation, cells were cotransfected with RNAi constructs together with pEGFP-F, a membrane-attached isoform

of GFP as a transfection marker. To quantify the distribution of PCM1 and ninein at the centrosome, a circle with 3-μm diameter was drawn centering on the γ-tubulin and defined as the area, including the centrosome. In all experimental groups, the immunointensity of PCM1 or ninein in the whole cell area vs centrosome area was quantified with Image J (http://rsb.info.nih.gov/ij/). The intensity ratio of the signal of more than 30 cells per group was analyzed in 3 independent experiments in a blinded manner. Statistical analyses were conducted with 1-way analysis of variance followed by post hoc testing. Values depicted are mean (SEM).

IN UTERO ELECTROPORATION AND IMMUNOHISTOCHEMISTRY

In utero electroporation was performed as described previously.[31,38] Validated shRNA plasmids in cell cultures (at a concentration of 4 μg/μL in 1-2 μL) were introduced directly into the ventricular zone by in utero electroporation of embryonic day 15 embryos as reported previously.[38] To confirm the specificity of the effects, dilution series of each RNAi plasmid in 1 to 2 μL were introduced, and their dose-correlated effects were confirmed. A GFP expression vector with CAG promoter was cotransfected with RNAi constructs at a concentration of 2 μg/μL. Coronal slices of the developing cerebral cortex were prepared at postnatal day 0 as described previously.[38] Briefly, the brains were fixed with 4% paraformaldehyde and sectioned with a cryostat at 20 μm on postnatal day 0. Green fluorescent images were captured after immunofluorescent staining with an anti-GFP antibody (dilution, 1:500). Nuclei were labeled with propidium iodide (Molecular Probes). Slice images were acquired with a confocal microscope (LSM 510; Zeiss and FV300; Olympus Optical, Center Valley, Pennsylvania).

QUANTITATIVE BIN ANALYSIS OF BRAIN SLICES

To quantify the pattern of migration, the numbers of GFP-positive cells in the developmental cerebral cortex, including the ventricular zone, the subventricular zone/intermediate zone, and the cortical plate, were counted from 3 independent sections. We quantified the RNAi effect on neuronal migration status by bin analysis, in which the developing cerebral cortex was divided into 10 equal spaces (10 bins) and the percentage of GFP-positive cells in each bin was determined. The numbers of neurons in each category from more than 5 independent experiments were counted in a blinded manner. Migration distance was defined as the relative distance of each cell migration (from the surface of the ventricle) to the radial thickness of the cerebral cortex where the cells were located. Image J was used for the assay. Statistical analyses were conducted with 1-way analysis of variance followed by post hoc testing. Values depicted are mean (SEM).

SEQUENCE ANALYSIS OF PCM1 IN PATIENTS WITH SZ

We analyzed DNA from 32 unrelated patients with SZ, from families that have been reported previously to have excess allele sharing among affected individuals at 8p22,[7] for exonic variations in PCM1. DNA samples were extracted according to standard protocol. Details about the clinical assessment of the samples are available in our previous study.[7] We also screened 219 white control samples, matched for ethnicity to the patients with SZ and evaluated for the absence of mental illnesses (*DSM-IV* criteria). For all the subjects (both controls and cases), we used 2 independent genotyping methods: first, we performed polymerase chain reaction and then bidirectional sequencing using BigDye Primer v.1 and the ABI377 sequencer (Applied Biosystems, Foster City, California), and second, we screened by custom TaqMan SNP genotyping assays (Applied Biosystems).

RESULTS

INTERACTION OF PCM1, DISC1, AND BBS PROTEINS AT THE CENTROSOME

To explore a possible functional relationship among PCM1, DISC1, and BBS proteins, we first tested whether these molecules could interact with each other. Exogenous protein interactions were tested by coimmunoprecipitation in HEK293 cells. HA-tagged PCM1 coprecipitated with myc-tagged DISC1 but not with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (**Figure 1**A). HA-tagged DISC1 coprecipitated with all the BBS proteins we tested that were tagged with myc (BBS1, BBS2, BBS4, BBS5, BBS6, BBS7, and BBS8) but not with GAPDH (Figure 1B). This result suggests that DISC1 might be an important component in the BBS common pathway. Our previous study had already demonstrated an interaction of BBS4 and PCM1 proteins at the centrosome.[21] Thus, we tested colocalization of DISC1, PCM1, BBS1, and BBS4 proteins at the centrosome in immature cortical neurons (Figure 1C). DISC1, BBS1, and BBS4 colocalized almost perfectly with γ-tubulin, an established centrosomal marker, whereas PCM1 localized as granular structures at and around the centrosome in a manner reminiscent of its distribution in fibroblasts and other cell types.[19]

PCM1, DISC1, AND BBS4 INTERACT WITH EACH OTHER THROUGH DISTINCT BINDING DOMAINS

To characterize DISC1 domains crucial for the interaction with PCM1 and BBS proteins, we expressed 3 HA-tagged DISC1 fragments in HEK293 cells. Endogenous BBS1, BBS4, and BBS8 coprecipitated commonly with the middle portion of DISC1 containing amino acids 349 to 600 (DISC1 [349-600]) but not the N-terminal (DISC1 [N-348]) nor the C-terminal DISC1 fragments (DISC1 [601-C]) (**Figure 2**A). By contrast, the N-terminal (DISC1 [N-348]) and C-terminal (DISC1 [601-C]) domains, distinct from the domain for BBS proteins, mediated the interaction between DISC1 and PCM1 (Figure 2A). The C-terminal domain of DISC1 for binding with PCM1 is distinct from the domain for NDEL1 binding, demonstrated by the interaction of PCM1 to DISC1 lacking the NDEL1 binding site (DISC1Δ[802-835]) (Figure 2B).[32] BBS4 is required for the recruitment of PCM1 to the centrosome.[21] We therefore focused on BBS4 for further analysis of the DISC1-PCM1-BBS protein interaction. The BBS4 protein is composed of 13 tandem tetratricopeptide repeat (TPR) motifs, flanked with short N- and C-terminal sequences. Sequential deletion of BBS4 protein from the N terminus indicated that HA-tagged DISC1 could interact with a BBS4

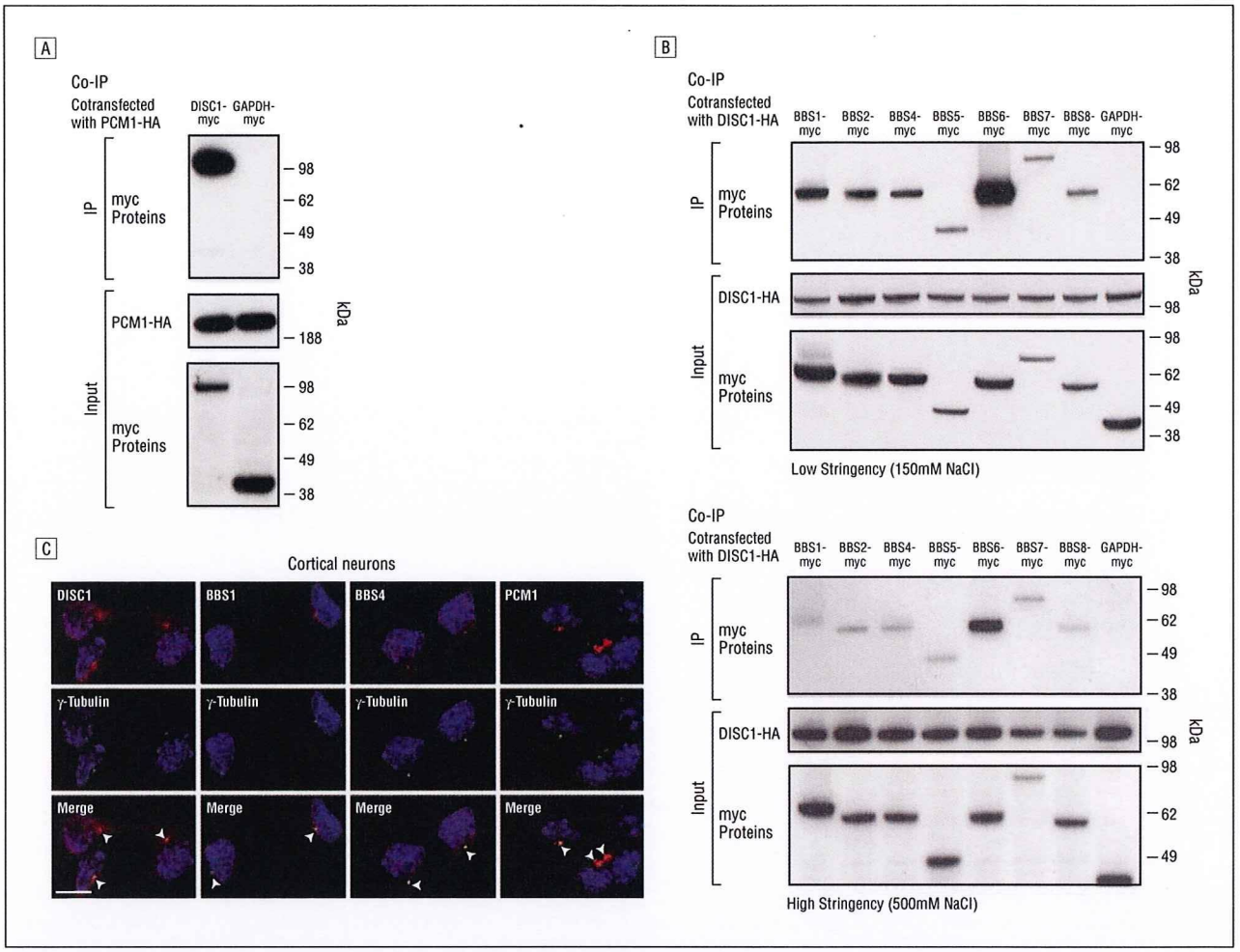

Figure 1. Pericentriolar material 1 (PCM1), disrupted-in-schizophrenia 1 (DISC1), and Bardet-Biedl syndrome (BBS) proteins interact, localizing with γ-tubulin at the centrosome. A, PCM1 interacts with DISC1. HA-tagged PCM1 (PCM1-HA) was coexpressed with myc-tagged DISC1 (DISC1-myc) in HEK293 cells. Cell extracts were immunoprecipitated with an anti-HA antibody. Immunoprecipitates (IPs)were analyzed by Western blotting with an anti-myc antibody (upper panel). The input of each protein is also shown (middle and bottom panels). GAPDH-myc indicates myc-tagged glyceraldehyde-3-phosphate dehydrogenase. B, BBS proteins interact with DISC1 in coimmunoprecipitation (Co-IP) in HEK293 cells. Myc-tagged BBS1 (BBS1-myc), 2, 4, 5, 6, 7, and 8 all bind to HA-tagged DISC1 (DISC1-HA). Consistent results were observed under both a low-stringency (150mM sodium chloride [NaCl]) washing condition (upper panels) as well as a high-stringency (500mM NaCl) washing condition (lower panels). The input of each protein is shown in the middle and bottom panels. C, Localization of BBS1, BBS4, DISC1, and PCM1 in immature cortical neurons at 3 days in vitro. Endogenous BBS1, BBS4, and DISC1 (red) are colocalized with γ-tubulin at the centrosome (arrowheads). Endogenous PCM1 (red) mainly occurs just adjacent to γ-tubulin with slight overlap with each other. Blue indicates the nucleus; green, γ-tubulin. Scale bar, 10 μm.

protein that maintains the portion from the second TPR to the C terminus but failed to bind to BBS4 once the second TPR was lost (Figure 2C). In contrast, the same sequential deletion of BBS4 for testing interaction with PCM1 revealed that deletion of the third TPR dramatically reduced the PCM1-BBS4 binding (Figure 2D). The domain of PCM1 for binding to DISC1 was tested by using 3 PCM1 fragments, indicating that the middle portion of PCM1 (amino acids 741-1420) was required for the PCM1-DISC1 interaction (Figure 2E), which, given that the C-terminal portion of PCM1 (amino acid 1913 to the C terminus) is required for binding to BBS4,[21] suggests that the PCM1-DISC1 interaction is discrete from the PCM1-BBS4 interaction. Overall, based on these pairwise binding data between the 3 proteins, we conclude that PCM1, DISC1, and BBS4 likely interact with each other through distinct binding domains (Figure 2F).

DISC1 AND BBS4 ACT SYNERGISTICALLY TO INFLUENCE RECRUITMENT OF PCM1 AND NINEIN TO THE CENTROSOME

We reported previously that DISC1 plays a role in recruiting dynein motor proteins, such as dynein intermediate chain and dynactin p150^{glued}, to the centrosome.[31] We also showed that BBS4 binds to p150^{glued} that is required for recruiting PCM1 to the centrosome.[21] Because PCM1 interacts with DISC1 and BBS4 through distinct domains, we hypothesized that DISC1 and BBS4 may act synergistically to recruit PCM1 to the centrosome. To test this idea, we used RNAi against each of DISC1, BBS4, and PCM1 (**Figure 3**A) and examined the effects in PC12 cells (Figure 3B). Knockdown expression of DISC1 reduced accumulation of PCM1 to the centrosome. Consistent with our previous findings in HeLa cells,[21] knockdown expression of BBS4 resulted in

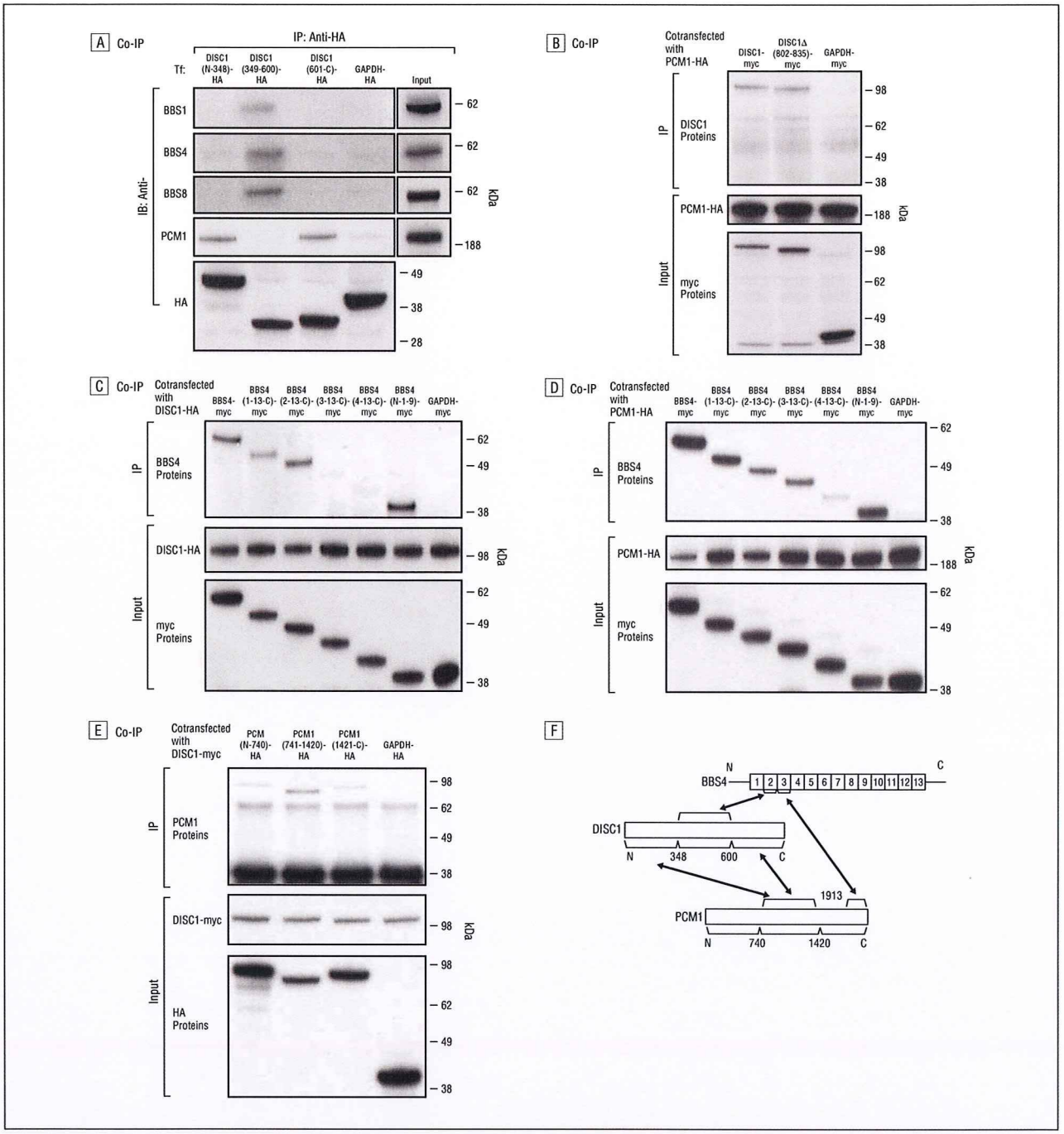

Figure 2. Pericentriolar material 1 (PCM1), disrupted-in-schizophrenia 1 (DISC1), and Bardet-Biedl syndrome 4 (BBS4) interact with each other through distinct binding domains. A, The middle portion of DISC1 (amino acids 349-600) is crucial for DISC1-BBS4 protein interaction. The N-terminal portion (amino acids 1-348) and the C-terminal portion (amino acids 601-854) of DISC1 are important for the DISC1-PCM1 binding. Three HA-tagged DISC1 protein fragments (DISC1 [N-348]-HA, DISC1 [349-600]-HA, and DISC1 [601-C]-HA) were expressed in HEK293 cells for coimmunoprecipitation (Co-IP) with an anti-HA antibody. The middle portion of DISC1, but not the N- nor C-terminal DISC1, binds to each of BBS1, 4, and 8, whereas the N- and C-terminal DISC1 bind to PCM1 (upper panels). The inputs of each protein are shown at the right and bottom panels. IB indicates antibodies used for Western blotting; GAPDH-HA, HA-tagged glyceraldehyde-3-phosphate dehydrogenase; Tf, transfection. B, The C-terminus domain of DISC1 for interaction with PCM1 is distinct from the NDEL1 binding domain of DISC1. Deletion of the DISC1-NDEL1 binding region (DISC1Δ[802-835]) had no effect on the interaction of DISC1 with PCM1. The inputs are shown in the middle and bottom panels. PCM1-HA indicates HA-tagged PCM1; DISC1-myc, myc-tagged DISC1; DISC1Δ(802-835)-myc, myc-tagged DISC1Δ(802-835); GAPDH-myc, myc-tagged GAPDH. IP, immunoprecipitation. C, The second tandem tetratricopeptide repeat (TPR) motif of BBS4 is crucial for the BBS4-DISC1 interaction. A series of myc-tagged BBS4 (BBS4-myc) truncation mutants were coexpressed with DISC1-HA in HEK293 cells for Co-IP with an anti-HA antibody. Deletion of the N-terminal region in BBS4 (1-13-C) and further deletion of the first TRP motif (BBS4 [2-13-C]) does not affect the BBS4-DISC1 interaction. By contrast, BBS4 mutants with further deletion of the second TRP motif (BBS4 [3-13-C] and BBS4 [4-13-C]) did not bind with DISC1. BBS4 lacking the C-terminal region (BBS4 [N-1-9]) binds to DISC1. The inputs are also shown (middle and bottom panels). D, The third TPR motif of BBS4 is important for the BBS4-PCM1 interaction. Deletion of the third TPR motif (BBS4 [4-13-C]) dramatically weakened the interaction of BBS4-myc with PCM1-HA. An anti-HA antibody was used for Co-IP. The inputs are shown in the middle and bottom panels. E, The middle portion of PCM1 is important for DISC1-PCM1 interaction. Myc-tagged DISC1 was coexpressed with PCM1-HA protein fragments in HEK293 cells for Co-IP with an anti-myc antibody. PCM1 (741-1420) has stronger binding affinity to DISC1 than PCM1 (N-740) and PCM1 (1421-C). The inputs of each protein are also shown in the middle and bottom panels. F, Schematic of DISC1, BBS4, and PCM1 interaction shows that these proteins may interact with each other through distinct binding domains. N indicates N terminal; C, C terminal.

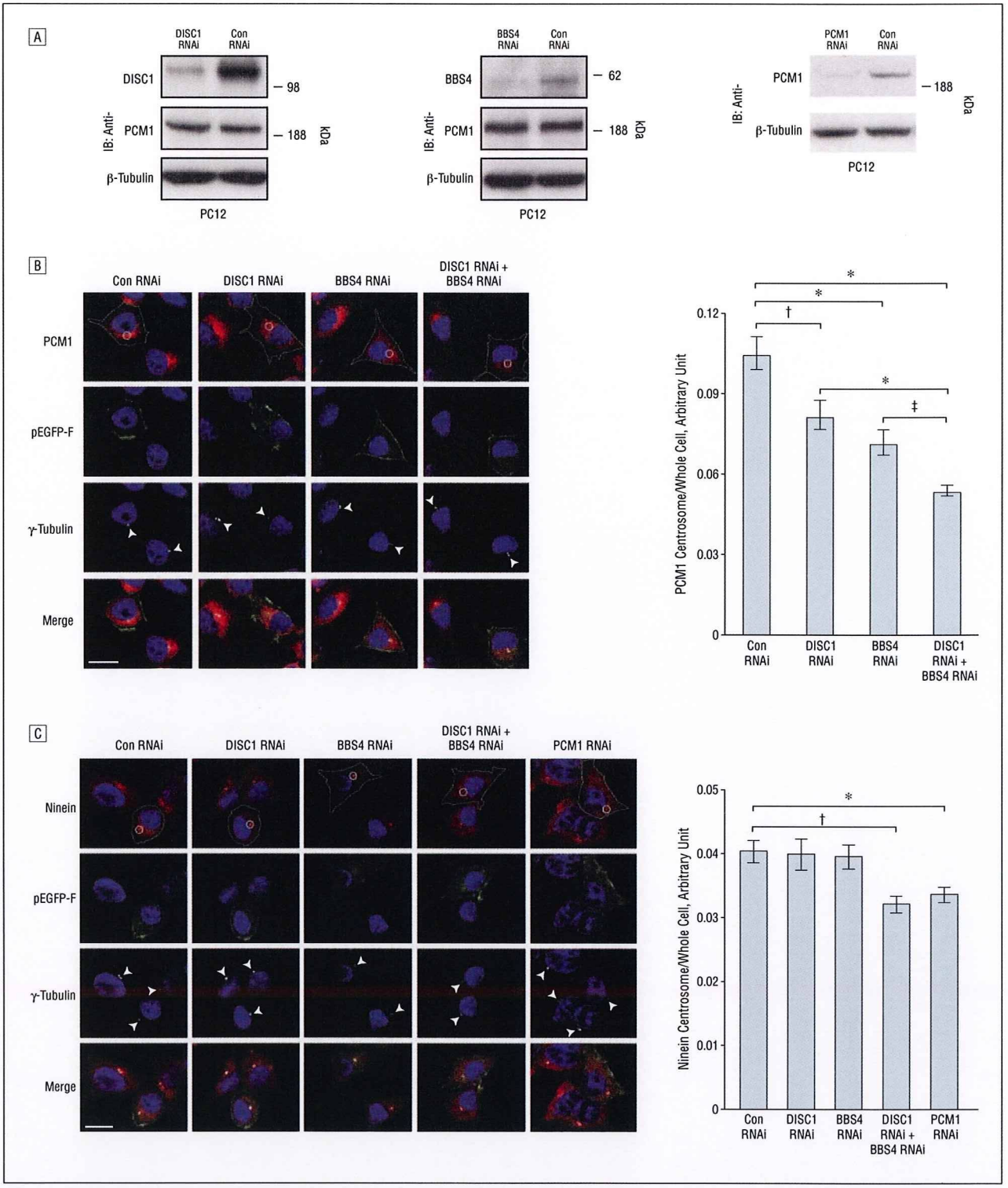

Figure 3. Synergistic effect of disrupted-in-schizophrenia 1 (DISC1) and Bardet-Biedl syndrome 4 (BBS4) on recruitment of pericentriolar material 1 (PCM1) and ninein to the centrosome. A, Efficient suppression of DISC1, BBS4, and PCM1 by RNA interference (RNAi). RNAi to DISC1, BBS4, and PCM1 suppresses 78%, 65%, and 78% of endogenous DISC1, BBS4, and PCM1 expression, respectively, in PC12 cells (top panels). RNAi to DISC1 or BBS4 does not affect the levels of endogenous PCM1 (middle panels). IB indicates antibodies used for Western blotting; Con RNAi, control RNAi. B, Suppression of DISC1 and BBS4 reduces accumulation of PCM1 to the centrosome in PC12 cells in a synergistic manner. To quantify the accumulation, immunointensity of PCM1 in the centrosome area (white circle) relative to that in the whole cell region surrounded by the green line was quantified. Bars represent means of each group of cells in 3 independent and blinded experiments (*$P<.001$, †$P<.01$, ‡$P<.05$). Error bars represent standard error of the mean. Representative images are shown. Blue indicates the nucleus; red, PCM1; green, pEGFP-F; white, γ-tubulin (also indicated by arrowheads). Scale bar, 10 μm. C, Accumulation of ninein at the centrosome is disturbed by synergistic application of DISC1 and BBS4 RNAi or PCM1 RNAi. Although neither application of DISC1 RNAi nor BBS4 RNAi leads to a significant effect on ninein, the synergistic application of both RNAis reduces accumulation of ninein to the centrosome, resembling the phenotype in the presence of RNAi to PCM1. To quantify the accumulation, immunointensity of ninein in the centrosome area (white circle) relative to that in the whole cell region surrounded by the green line was quantified. Bars represent means of each group of cells in 3 independent and blinded experiments (*$P<.005$, †$P<.001$). Error bars represent standard error of the mean. Representative images of PC12 cells are shown. Blue indicates the nucleus; red, ninein; green, pEGFP-F; white, γ-tubulin (also indicated by arrowheads). Scale bar, 10 μm.

decreased enrichment of PCM1 to the centrosome. Of most importance, knockdown of both DISC1 and BBS4 had a significantly stronger influence on the distribution of PCM1 than either single knockdown, consistent with the hypothesis that DISC1 and BBS4 cooperate to regulate the recruitment of PCM1 to the centrosome. PCM1 plays a role in further recruiting other centrosomal proteins, such as ninein.[18] We therefore tested whether DISC1 and BBS4 also influence PCM1-associated molecular recruitment to the centrosome in a synergistic manner by examining the effects of RNAi on DISC1, BBS4, and PCM1 with respect to the localization of ninein (Figure 3C). Knockdown expression of either PCM1 or both DISC1 and BBS4 similarly reduced the amount of ninein at the centrosome in PC12 cells.

KNOCKDOWN EXPRESSION OF PCM1, DISC1, AND BBS4 LEADS TO NEURONAL MIGRATION DEFECTS IN THE DEVELOPING CEREBRAL CORTEX IN VIVO

To evaluate the physiological relevance of our findings, we tested the influence of PCM1, DISC1, and BBS4 in vivo by suppressing their expression in the developing cerebral cortex by in utero gene transfer.[31,38] Embryos were electroporated with shRNA at embryonic day 15, and the effect of suppression was evaluated by immunohistochemistry, followed by a bin distribution analysis of neurons at postnatal day 0 (**Figure 4** and the eFigure, http://www.archgenpsychiatry.com). Brain slices electroporated with control RNAi together with a GFP marker showed that 25% of GFP-labeled cells completed migration through the cortical wall and formed the superficial layers of the cortex that corresponded to bins 9 and 10. By contrast, in brain slices electroporated with DISC1 RNAi, radial neuronal migration was significantly delayed, as reported previously.[31] Suppression of either BBS4 or PCM1 phenocopied the DISC1 phenotype in neuronal migration. Importantly, concomitant suppression of both DISC1 and BBS4 led to significantly more severe impairment in migration compared with that of DISC1 alone.

A CANDIDATE PATHOGENIC PCM1 MUTATION IN AN SZ FAMILY

Our data have shown that DISC1 and BBS4 are necessary for targeting PCM1 to the centrosome, with concomitant targeting effects for ninein and likely other molecules for their transport to the centrosome, in a PCM1-dependent manner. Consistent with this notion, a recent study reported association of PCM1 haplotypes with SZ and volumetric defects in the gray matter of the orbitofrontal cortex,[2] although a causative mutation has not been found to date. We therefore examined the *PCM1* gene for mutations in a SZ cohort by focusing on the coding region of the gene, since variations there would be less challenging to interpret. An emerging hypothesis in the field of SZ is that a portion of the genetic load may be contributed by rare, possibly strong, alleles.[39] We therefore focused on testing primarily for rare alleles by performing direct bidirectional sequencing of the 39 exons and flanking splice sites in 32 probands.[7] In addition to synonymous single-nucleotide polymorphisms (SNPs) that are unlikely to affect the *PCM1* transcript or protein, we found 2 previously known missense mutations in our cohort (**Table**). The first allele, SNP rs370429 (encoding a T1543I change), has been reported to be associated with SZ.[2] Different from the data by Gurling et al,[2] we failed to find any association between this SNP and SZ, probably because of our small sample size. Likewise, for a second missense allele (rs412750; S159N at the amino acid level), we failed to detect allelic association, which is consistent with previous work.[2] The genotypic frequency of this variant is significantly different in patients with SZ (Fisher exact test, $P=.01$) (Table). We think it unlikely that this represents a genotyping error inherent to the assay, since we saw no deviation from Hardy-Weinberg equilibrium in the large control group ($P=.35$); nonetheless, to confirm this result, we regenotyped all individuals from both cases and controls with a TaqMan assay. There was no genotyping error observed since the genotypes were attained in 2 different methods that had the same result. We found additional evidence of a relationship between *PCM1* and SZ. In 1 individual, we found a heterozygous 4057G→T mutation that introduced a premature termination codon (E1353X) in exon 24, which leads to either truncation of the protein, eliminating 672 residues from the C terminus, or, more likely, triggers the nonsense-mediated decay by virtue of the introduction of a premature termination codon.[40] This allele was not present in any of 219 ethnically matched controls, whereas segregation analysis showed that the E1353X allele was also present in the heterozygous state in the affected mother and the affected sibling of the proband but not in the unaffected members of the maternal and paternal sibship (**Figure 5**). This result supports a possible role for this PCM1 loss-of-function allele for SZ in this family. Clearly, a mutation in a single family with a priori linkage to the 8p region is not sufficient to generalize the causal link between PCM1 loss of function and SZ. However, the combination of this result with the previous association of PCM1 with SZ[2] and, importantly, the biochemical relationship of PCM1 to DISC1 as it pertains to key neurodevelopmental processes pose a compelling argument.

COMMENT

In the present study, we provide 2 lines of evidence that support a role for the centrosome in the pathology of SZ. Biological data indicate a centrosomal pathway that includes the PCM1, DISC1, and BBS proteins playing a role in proper cortical development. Genetic data further confirm the notion that PCM1 is a risk factor for SZ by providing a nonsense mutation that segregates with SZ spectrum psychosis in a pedigree.

We found that DISC1 interacts with several BBS proteins (BBS1, BBS2, BBS4, BBS5, BBS6, BBS7, and BBS8) and that DISC1 may possess a common binding domain for at least BBS1, BBS4, and BBS8. Our data on the interaction of DISC1 with all BBS proteins tested suggest

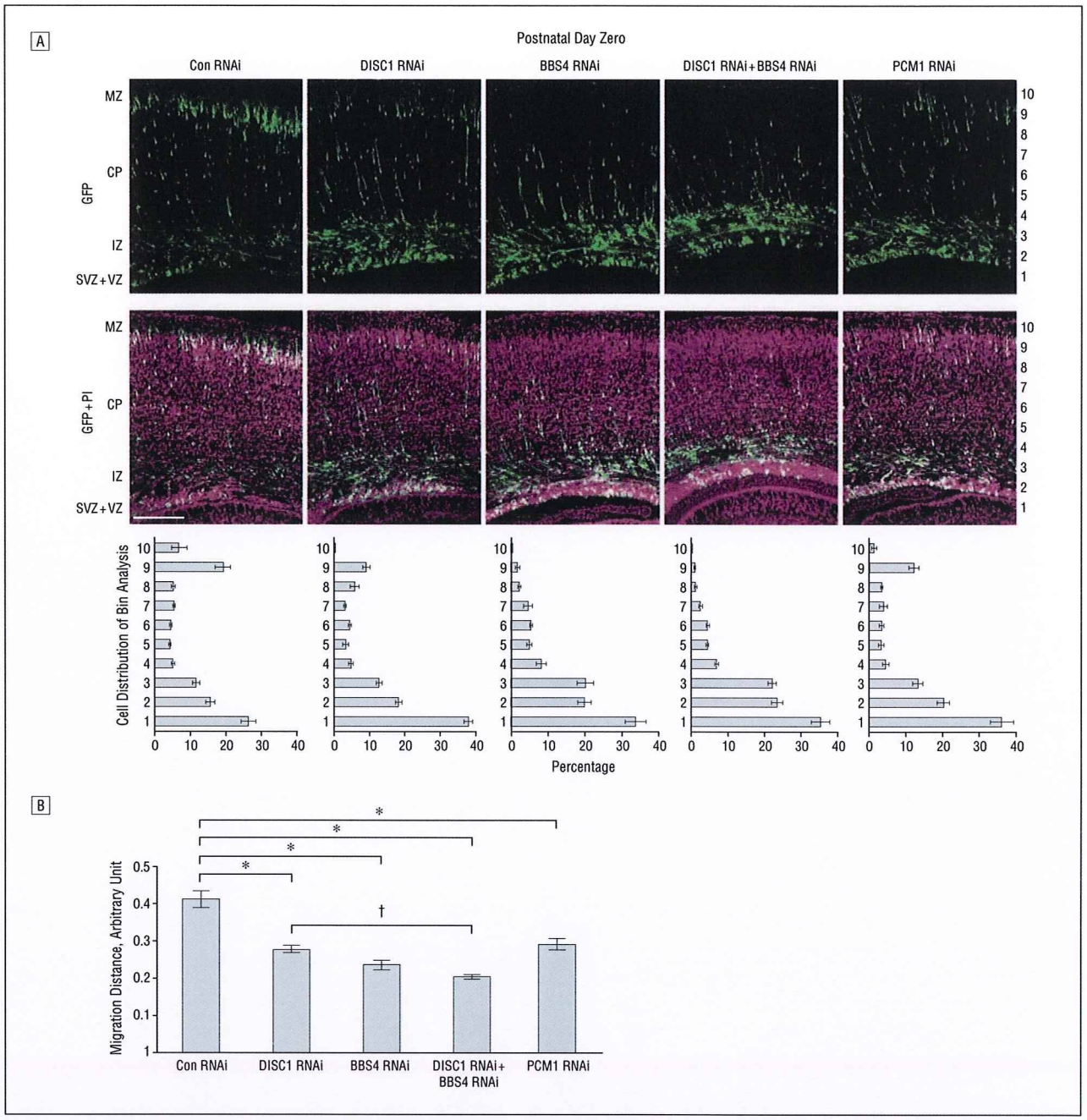

Figure 4. Knockdown of disrupted-in-schizophrenia 1 (DISC1), Bardet-Biedl syndrome 4 (BBS4), and pericentriolar material 1 (PCM1) leads to neuronal migration defects in the developing cerebral cortex. A, RNA interference (RNAi) constructs and green fluorescent protein (GFP) expression vectors were electroporated into the ventricular zone (VZ) at embryonic day 15 and analyzed at postnatal day 0. In brains with control RNAi (Con RNAi), 40% of GFP-labeled cells exited the VZ, and 25% of GFP-labeled cells completed migration and formed the superficial layers of the cortex that correspond to bins 9 and 10. By contrast, only less than 15% of GFP-positive cells reached the superficial layers in brain slices with DISC1 RNAi, BBS4 RNAi, or PCM1 RNAi, with the majority of GFP-positive cells remaining in the intermediate zone (IZ), subventricular zone (SVZ), and VZ. Green indicates cells cotransfected with GFP and RNAi constructs; purple, propidium iodide (PI); Con RNAi, control RNAi; CP, cortical plate; MZ, marginal zone. Scale bar, 100 μm. B, A migration distance is shown. Silencing of DISC1, BBS4, or PCM1 induces delayed radial migration (*$P<.001$). Silencing of both DISC1 and BBS4 expression leads to a more severe defect compared with that with either DISC1 RNAi or BBS4 RNAi. †$P<.05$. Values are given as mean (SEM).

that DISC1 may regulate the common pathway involving BBS. We also found that PCM1, DISC1, and BBS4 interact with each other at least through "distinct" binding domains. As a future perspective, minimal binding domains for each protein interaction will be determined by a series of deletion mutants as well as full-length proteins that have specific deletion of identified binding domains.

Our data also suggest that DISC1 and BBS4 target PCM1 and the associated cargo protein ninein synergistically to the centrosome. It remains to be determined, however, whether these proteins interact directly and whether other centrosomal proteins, such as centrin and pericentrin, are also regulated by the interaction of DISC1 with BBS4. One question is how either DISC1 RNAi alone or BBS4 RNAi alone shows minor effects on the accu-

Table. List of *PCM1* Variants in Our Cohort of Patients With SZ and Controls[a]

Exon	Amino Acid Change	Nucleotide Change	Genotype Frequency, % (Sample Size)					
			AA in SZ	Aa in SZ	aa in SZ	AA in Control	Aa in Control	aa in Control
5	S159N[b]	476GT→AC/AT (rs412750)	75 (24)	9.4 (3)	15.6 (5)	57.5 (126)	32.9 (72)	9.6 (21)
24	E1353X[c]	4057G→T	96.9 (31)	3.1 (1)	0	100 (219)	0	0
28	T1543I	4628C→T (rs370429)	93.8 (30)	6.2 (2)	0	92.2 (202)	7.8 (17)	0

Abbreviations: A, corresponds to the major allele found in NM_006197; a, corresponds to the minor allele found in NM_006197; SZ, schizophrenia.
[a]The distribution of genotypes for the controls for both rs412750 and rs370429 did not deviate significantly from Hardy-Weinberg equilibrium ($P = .35$ and .91, respectively).
[b]There are no allelic associations between rs412750 and SZ in this sample set, although the genotypic frequency of S159N is significantly different in patients with SZ (Fisher exact test, $P = .01$) compared with controls.
[c]The nonsense mutation (E1353X) was found in a single patient with SZ and no controls.

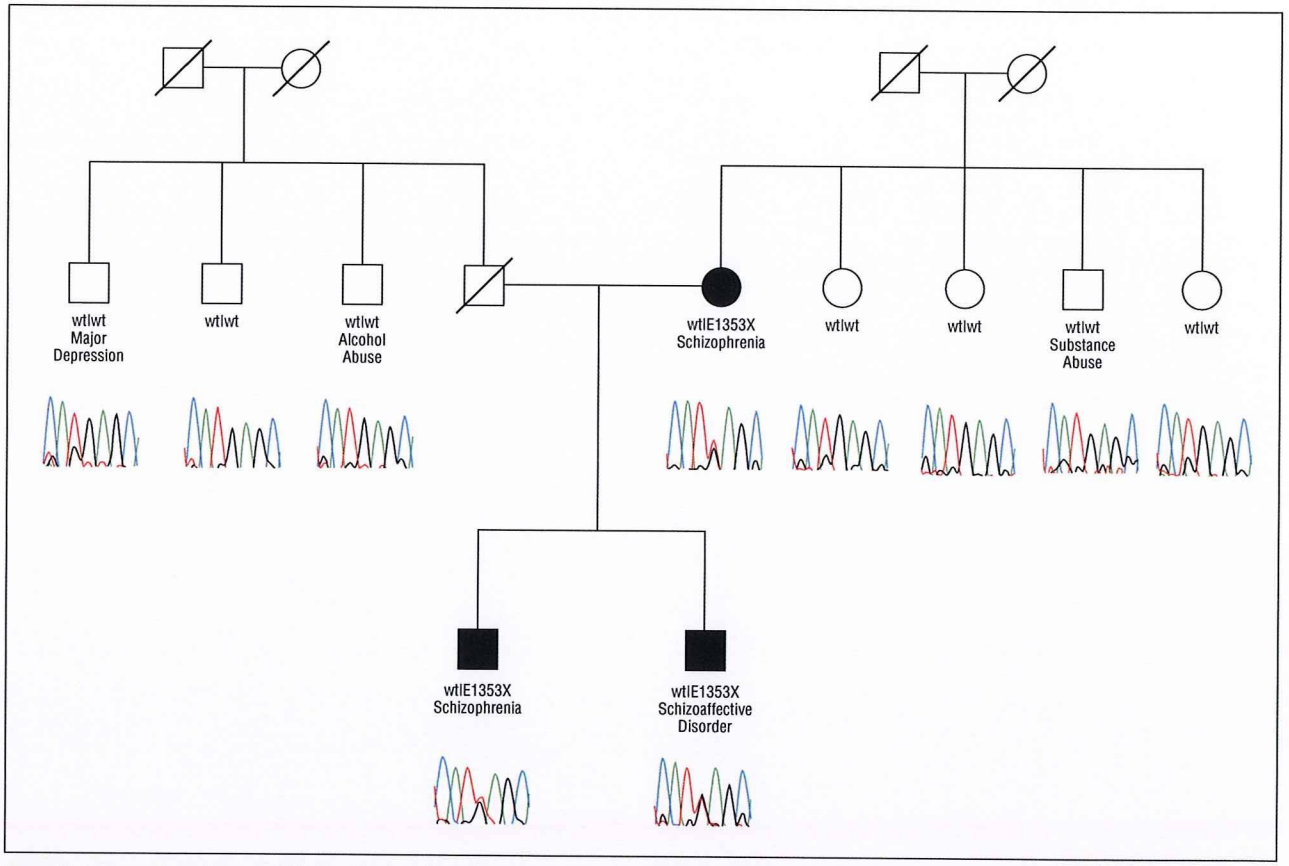

Figure 5. A nonsense mutation in *PCM1* in a family with schizophrenia and schizoaffective disorder. Mutation analysis of a white family. JHU37007 shows a heterozygous 4057G→T mutation in exon 24 of *PCM1*, introducing a premature termination codon (E1353X); genotypes are shown below each individual, as are sequence traces. The psychiatric phenotype (if any) of each family member is also shown. wt Indicates wild type.

mulation of ninein to the centrosome, whereas such treatment affects the localization change of PCM1. This may be because accumulation of ninein to the centrosome is affected only when the levels of PCM1 at the centrosome fall below the threshold by the synergistic effects of both DISC1 and BBS4 RNAi.

Neuronal migration defects were observed when we knocked down DISC1, BBS4, or PCM1 in the developing cerebral cortex, which is consistent with the notion of the role of the centrosome in corticogenesis. We believe that interpretation of the data should be viewed with caution, however, because the knockdown of these proteins may potentially affect their other cellular functions related to neuronal migration. For instance, DISC1 is a multifunctional protein localized at the centrosome, mitochondria, postsynaptic densities, and the nucleus.[3] Future studies might address this issue by coelectroporation of RNAi and expression constructs of DISC1 in which coexpression of wild-type DISC1 rescues the phenotypes resulting from DISC1 RNAi, whereas mutant DISC1 selectively deficient in the binding domains for BBS4 or PCM1 may not rescue the pathology. That PCM1 knockdown has a weaker influence on migration defects than does coknockdown of BBS4 and DISC1 might be explained by considering that knockdown of DISC1 and BBS4 may potentially affect their other cellular functions related to neuronal migration,