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,\(^3,8\) 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-
mainly 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-BBS 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.20,21,23,30 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'-GGCAACACTGTAAGTGCC-3'; PCM1 RNAi, 5'-TCAGCTTTGATTCCTCAAG-3'; and BBS4 RNAi, 5'-GGAGCTATCGCCTGCTAA-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 (300 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 IgP 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 500 mM. 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). Harvested 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 0.3% 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, 1:300; 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, Göttingen, Germany) was used for epifluorescence 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

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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 V/9253; H9004). Image J was 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, distinct from the domain for BBS proteins, mediating the interaction between DISC1 and PCM1 (Figure 2A). 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.31 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

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 1A). 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 localized 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

PCML, 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 2A). 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.31 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
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, 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 RECRUITEMENT 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 p150glued, to the centrosome.\(^{31}\) We also showed that BBS4 binds to p150\(^{\text{glued}}\) 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 3A) 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...
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, BBS4, and BBS8, whereas the N- and C-terminal DISC1 bind to PCM1 (upper panels). The inputs of each protein are also 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 (DISC1 [349-600]-HA, and DISC1 [601-C]-HA) did not affect the interaction of DISC1 with PCM1. The middle portion of DISC1, but not the N- nor C-terminal DISC1, binds to each of BBS1, BBS4, and BBS8, whereas the N- and C-terminal DISC1 bind to PCM1 (upper panels). The inputs of each protein are also shown at the right and bottom panels. 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-myc antibody. 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.\textsuperscript{18} We therefore tested whether DISC1 and BBS4 also influence PCM1-associated molecular recruitment to the centrosome in a synergetic 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.\textsuperscript{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 (\textbf{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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{30} 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.\textsuperscript{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 (\textbf{Table}). The first allele, SNP rs370429 (encoding a T15431 change), has been reported to be associated with SZ.\textsuperscript{2} Different from the data by Gurling et al,\textsuperscript{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.\textsuperscript{2} The genotypic frequency of this variant is significantly different in patients with SZ (Fisher exact test, \(P = .01\)) (\textbf{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.\textsuperscript{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 (\textbf{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\textsuperscript{2} and, importantly, the biochemical relationship of PCM1 to DISC1 as it pertains to key neurodevelopmental processes pose a compelling argument.

\textbf{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
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 accum-
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. 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.

### Table. List of PCM1 Variants in Our Cohort of Patients With SZ and Controls

<table>
<thead>
<tr>
<th>Exon</th>
<th>Amino Acid Change</th>
<th>Nucleotide Change</th>
<th>Genotype Frequency, % (Sample Size)</th>
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<td></td>
<td></td>
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<td>AA in SZ</td>
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<tr>
<td>5</td>
<td>S159N&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>T1543I</td>
<td>4628C→T (rs370429)</td>
<td>93.8 (30)</td>
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Abbreviations: A, corresponds to the major allele found in NM_006197; a, corresponds to the minor allele found in NM_006197; SZ, schizophrenia.

<sup>a</sup>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).

<sup>b</sup>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.

<sup>c</sup>The nonsense mutation (E1353X) was found in a single patient with SZ and no controls.

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Figure 5. A nonsense mutation in PCM1 in a family with schizophrenia and schizoaffective disorder. Mutation analysis of a white family, JHUS7007 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.
whereas PCM1 has more restricted function associated with the centrosome.

In addition to DISC1 presenting the most compelling genetic argument for participation in SZ,2,3 2 centrosome-related genes, PCM1 and NDE1, have also been proposed as potential SZ susceptibility genes.2,3 Nonetheless, it is difficult to identify the causal mutation(s), in part because of allelic heterogeneity. Herein, we have identified a bona fide loss of function mutation (a nonsense allele), which segregates with SZ spectrum psychosis. Given our functional data, we speculate that haploinsufficiency at the PCM1 locus will lead to compromised, but not abolished, PCM1-associated centrosomal functions, which can potentially lead to more subtle neurodevelopmental effects. Notably, patients with SZ who showed an association with PCM1 had gray matter deficits in the orbitofrontal cortex.2 Lesions in this brain region are likely to compromise mechanisms that support reward-related processes and motivated behaviors.23 These are consistent with the finding that families with evidence for linkage to 8p21-22 had significantly more affective deterioration, poorer outcome, more thought disorder, and fewer depressive symptoms than did affected individuals from non–8p21-22-linked families.23 Taken together, we speculate that genetic variations of PCM1 are associated with a subtype of SZ that primarily displays negative symptoms, referred to commonly as deficit SZ.43 Nonetheless, the chromosome locus of 8p21-22 has linked to order, and fewer depressive symptoms than did affected individuals from non–8p21-22-linked families.42 Taken together, we speculate that genetic variations of PCM1 are associated with a subtype of SZ that primarily displays negative symptoms, referred to commonly as deficit SZ.43 Nonetheless, the chromosome locus of 8p21-22 has linked to both SZ and mood disorders.44,45 Thus, PCM1 may also be a risk factor for affective disorders by participating in some aspects of the pathophysiology of the diseases.

Our data also suggest that BBS genes might also be potential candidates contributing susceptibility alleles for major mental illnesses. Consistent with this notion, recent epidemiological findings have shown that patients with BBS are at least twice as likely to develop SZ compared with the general population and have various psychiatric conditions at high prevalence (>30%).22,23 Intriguingly, BBS and SZ also share other phenotypes, such as olfaction deficits, obesity, and type 2 diabetes mellitus.22,23,28,44-46

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Additional Information: The eFigure is available at http://www.archgenpsychiatry.com.

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