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Interaction between FEZ1 and DISC1 in Regulation of Neuronal Development and Risk for Schizophrenia

Eunchai Kang,[1,2] Katherine E. Burdick,[6,7,8,10] Ju Young Kim,[1,3] Xin Duan,[1,4] Junjie U. Guo,[1,4] Kurt A. Sailor,[1,4] Dhong-Eun Jung,[1] Sundar Ganesan,[9] Sungkyung Choi,[1] Dennis Pradhan,[1,4] Bai Lu,[9] Dimitrios Avramopoulos,[2,5] Kimberly Christian,[1,3] Anil K. Malhotra,[6,7,8] Hongjun Song,[1,2,3,4] and Guo-li Ming[1,3,4,*]

[1]Institute for Cell Engineering
[2]Pre-doctoral Training Program in Human Genetics
[3]Department of Neurology
[4]The Solomon H. Snyder Department of Neuroscience
[5]Institute of Genetic Medicine
Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[6]Divison of Psychiatry Research, The Zucker Hillside Hospital, North Shore–Long Island Jewish Health System, Glen Oaks, NY 11004, USA
[7]Department of Psychiatry, Albert Einstein College of Medicine, New York, NY 10461, USA
[8]The Feinstein Institute for Medical Research, Manhasset, NY 11004, USA
[9]Genes, Cognition and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, USA
[10]Present address: Department of Psychiatry and Neuroscience, Mount Sinai School of Medicine, New York, NY 10029, USA
*Correspondence: gming1@jhmi.edu
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SUMMARY

Disrupted-in Schizophrenia 1 (*DISC1*), a suscepti-bility gene for major mental disorders, encodes a scaffold protein that has a multifaceted impact on neuronal development. How DISC1 regulates different aspects of neuronal development is not well understood. Here, we show that Fasciculation and Elongation Protein Zeta-1 (FEZ1) interacts with DISC1 to synergistically regulate dendritic growth of newborn neurons in the adult mouse hippo-campus, and that this pathway complements a parallel DISC1-NDEL1 interaction that regulates cell positioning and morphogenesis of newborn neurons. Furthermore, genetic association analysis of two independent cohorts of schizophrenia patients and healthy controls reveals an epistatic interaction between *FEZ1* and *DISC1*, but not between *FEZ1* and *NDEL1*, for risk of schizophrenia. Our findings support a model in which DISC1 regu-lates distinct aspects of neuronal development through its interaction with different intracellular partners and such epistasis may contribute to increased risk for schizophrenia.

INTRODUCTION

An abiding principle of brain organization holds that the precise synaptic connectivity of neuronal networks determines brain functions. Conversely, pathological disturbances of this neuronal and synaptic patterning may contribute to the symp-tomatology of many neurological and psychiatric illnesses. Therefore, understanding molecular mechanisms that regulate neuronal development and connectivity can generate insight into the processes that govern the functional integrity of the developing and adult brain. In the hippocampus of the adult mammalian brain, new neurons are continually generated from neural stem cells throughout the lifespan of the organism (Lledo et al., 2006; Ming and Song, 2005; Zhao et al., 2008). Adult neurogenesis recapitulates the complete process of embryonic neuronal development, including proliferation and fate specifica-tion of neural progenitors, morphogenesis, axon and dendritic growth, migration, and synapse formation of neuronal progeny (Duan et al., 2008; Ming and Song, 2011). Many signaling path-ways play conserved roles during embryonic and adult neuro-genesis and disruption of many of these same pathways have also been implicated in the etiology of psychiatric disorders (Harrison and Weinberger, 2005; Kempermann et al., 2008).

There is a growing body of evidence demonstrating a conver-gent effect of genetic mutations that both confer susceptibility to psychiatric diseases and result in dysregulation of neuronal development, supporting a neurodevelopmental origin of these diseases. One prominent example of this genetic convergence is disrupted in schizophrenia-1 (*DISC1*), a gene initially identified at the breakpoint of a balanced (1;11) (q42;q14) chromosome translocation in a large Scottish family that segregates with schizophrenia and other major mental disorders (Blackwood et al., 2001; Millar et al., 2000). Additional linkage studies with *DISC1* mutations further support its role in influencing risk for psychosis and autistic spectrum disorders (Chubb et al., 2008). Functional studies in animal models suggest that DISC1 plays a multifaceted role in both embryonic and postnatal neurogene-sis in vivo. Exogenous manipulation of DISC1 results in a spec-trum of neuronal abnormalities, depending on the timing and anatomical locus of perturbation. During embryonic cortical development, knockdown of DISC1 in E13 embryos accelerates cell cycle exit and neuronal differentiation (Mao et al., 2009), whereas knockdown at E14.5 leads to inhibition of neuronal migration and disorganized dendritic arbors (Kamiya et al.,

2005). During adult hippocampal neurogenesis, suppression of DISC1 also leads to decreased proliferation of neural progenitors (Mao et al., 2009) and an array of neurodevelopmental defects in newborn dentate granule cells, including soma hypertrophy, mispositioning, impaired axonal targeting, and accelerated dendritic growth and synaptogenesis (Duan et al., 2007; Faulkner et al., 2008; Kim et al., 2009).

The signaling mechanisms by which DISC1 regulates neurogenesis in vivo have just begun to be explored. For example, DISC1 regulates proliferation of neural progenitors through interaction with GSK3β (Mao et al., 2009), whereas it regulates development of newborn dentate granule cells through direct interaction with KIAA1212/Girdin in the hippocampus (Enomoto et al., 2009; Kim et al., 2009). NDEL1 (nuclear distribution gene E-like homolog 1) also directly interacts with DISC1 (Morris et al., 2003; Ozeki et al., 2003). Knockdown of NDEL1 in newborn neurons in the adult hippocampus leads to primary defects in neuronal positioning and appearance of ectopic dendrites, representing some, but not all, of phenotypes observed with DISC1 suppression (Duan et al., 2007). This result suggests the existence of additional mechanisms by which DISC1 regulates other aspects of neuronal development. Indeed, early biochemical and yeast two-hybrid screens have identified a large number of DISC1 binding partners, many of which are known to be involved in neurodevelopmental processes (Camargo et al., 2007). While these studies established DISC1 as a scaffold protein, the functional role of the majority of these potential interactions in neuronal development remains to be demonstrated in vivo. Understanding mechanisms by which DISC1 differentially regulates distinct neurodevelopmental processes through its binding partners may reveal how dysfunction of DISC1 contributes to a wide spectrum of psychiatric and mental disorders.

Fasciculation and Elongation Protein Zeta-1 (FEZ1) is one of the first identified binding partners of DISC1 (Miyoshi et al., 2003). FEZ1 is a mammalian ortholog of the *Caenorhabditis elegans* UNC-76 protein, thought to be involved in nerve growth and fasciculation (Bloom and Horvitz, 1997; Kuroda et al., 1999). FEZ1 expression is developmentally regulated and appears to be abundant in the adult mouse dentate gyrus (Miyoshi et al., 2003; Sakae et al., 2008). In vitro, FEZ1 colocalizes with DISC1 at neuronal growth cones and regulates neurite outgrowth of PC12 cells (Miyoshi et al., 2003). The role of FEZ1 in mammalian neuronal development in vivo is not well understood. *Fez1* null mice exhibit hyperactivity and enhanced responsiveness to psychostimulants (Sakae et al., 2008), supporting a potential contribution of *FEZ1* dysfunction to schizophrenia. Single nucleotide polymorphism (SNP) and haplotype association analyses of the *FEZ1* locus with schizophrenia have demonstrated a positive association in one cohort of patients (Yamada et al., 2004), but not in others (Hodgkinson et al., 2007; Koga et al., 2007; Nicodemus et al., 2010; Rastogi et al., 2009). Interestingly, there is a significant reduction of *FEZ1* mRNA in both hippocampus and dorsolateral prefrontal cortex of schizophrenia patients and an association of the *DISC1* genotype and *FEZ1* mRNA levels (Lipska et al., 2006). These findings raise the possibility that FEZ1 and DISC1 may cooperate to regulate both neuronal development and risk for schizophrenia.

In the present study, we used adult mouse hippocampal neurogenesis as an in vivo cellular model to dissect signaling mechanisms by which DISC1 regulates different aspects of neuronal development. We showed that interaction between FEZ1 and DISC1 regulates dendritic development of newborn dentate granule cells in the adult brain. This functional association complements the parallel DISC1-NDEL1 interaction, which regulates positioning and morphogenesis of newborn neurons. Biochemically, endogenous DISC1 interacts with both FEZ1 and NDEL1, whereas FEZ1 and NDEL1 do not appear to interact without DISC1. Furthermore, genetic association analyses in two clinical cohorts reveal an epistatic interaction between *FEZ1* and *DISC1*, but not between *FEZ1* and *NDEL1*, for an increased risk for schizophrenia. Together, our findings support a model in which DISC1 interacts with different partners to regulate distinct aspects of neuronal development and epistatic interactions between *DISC1* and these genes may exacerbate neurodevelopmental deficits and confer an increased risk for schizophrenia.

RESULTS

Regulation of New Neuron Development by FEZ1 in the Adult Brain

To explore signaling pathways underlying DISC1-dependent regulation of neuronal development, we generated retroviral vectors coexpressing GFP and specific short-hairpin RNAs (shRNAs) against mouse *fez1* (see Experimental Procedures). We first examined their efficacy in knocking down the expression of endogenous FEZ1 in cultured adult mouse neural progenitors, which expressed FEZ1 during the proliferation state and after induced neuronal differentiation (see Figure S1A available online). Two shRNAs against mouse *fez1* (shRNA-F1 and shRNA-F2), but not a control shRNA (shRNA-C1) (Ma et al., 2008), were very effective in knocking down the expression of endogenous FEZ, but not DISC1 or NDEL1, at the protein level (Figure 1A; Figure S1B).

To assess the potential function of FEZ1 in regulating development of newborn neurons in the adult brain, we stereotaxically injected retroviruses coexpressing shRNA and GFP into the dentate gyrus of the adult mice brain. GFP$^+$ newborn neurons were examined with confocal microscopy at 14 days postinjection (dpi). When compared with GFP$^+$ neurons expressing shRNA-C1, there was a significant increase in the soma size of GFP$^+$ neurons expressing either shRNA-F1 or shRNA-F2 (Figure 1B). Furthermore, GFP$^+$ neurons expressing either shRNA-F1 or shRNA-F2 exhibited accelerated dendritic development with significant increases in both total dendritic length and complexity as shown by the Sholl analysis (Figures 1C–1E). Interestingly, increased dendritic growth and soma hypertrophy have also been observed with DISC1 knockdown in these newborn dentate granule cells in the adult hippocampus (Duan et al., 2007). On the other hand, GFP$^+$ neurons with FEZ1 knockdown did not exhibit ectopic primary dendrites, aberrant neuronal positioning (Figure S1C), or mossy fiber axonal mistargeting (Figure S1D), other characteristic defects that result from DISC1 knockdown (Duan et al., 2007; Faulkner et al., 2008; Kim et al., 2009). Thus, FEZ1 knockdown leads to a specific subset of,
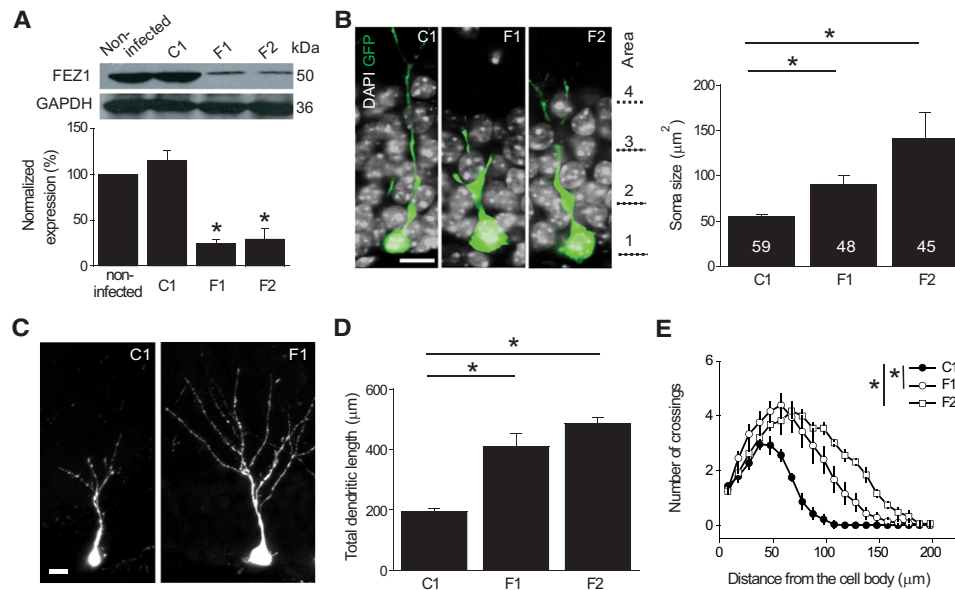
Figure 1. Role of FEZ1 in Regulating Development of Newborn Neurons in the Adult Mouse Dentate Gyrus

(A) Validation of the efficacy of shRNAs against mouse *fez1*. Retroviruses expressing shRNAs were used to infect mouse adult neural progenitors in culture and equal amount of samples from cell lysates were subject to western blot for FEZ1 and GAPDH. Shown are sample western blot analysis and quantification after expression of shRNA-control (C1), shRNA-FEZ1#1 (F1), or shRNA-FEZ1#2 (F2). Values represent mean ± SEM (n = 3; *p < 0.01; ANOVA).

(B) Morphological development of newborn neurons in the adult brain. Shown are sample confocal single section images of GFP and DAPI at 14 days post-injection (dpi) of retroviruses coexpressing GFP and shRNA in the adult dentate gyrus. The domains of cell body localization are indicated along the sample images (Area 1: inner granule cell layer; Area 2: middle granule cell layer; Area 3: outer granule cell layer; Area 4: molecular layer). Scale bar: 10 μm. Also shown is a summary of the soma size of GFP$^+$ cells under different conditions. Numbers associated with each bar graph refer to the total number of GFP$^+$ neurons analyzed from at least four animals under each condition. Values represent mean ± SEM (*p < 0.01; ANOVA).

(C–E) Dendritic development of newborn neurons in the adult dentate gyrus. Shown in (C) are sample confocal projection images of GFP$^+$ neurons at 14 dpi. Scale bar: 10 μm. Also shown are summary of the total dendritic length (D) and Sholl analysis of dendritic complexity (E) of GFP$^+$ newborn neuron expressing different shRNAs. The same sets of GFP$^+$ cells as in (B) were analyzed. Values represent mean ± SEM (*p < 0.01; ANOVA).

but not all, developmental defects observed in newborn neurons with DISC1 knockdown during adult neurogenesis.

Rescue of Development Defects from FEZ1 Knockdown by Wild-Type FEZ1 Expression

The similarity of phenotypes from two shRNAs against different regions of the *fez1* gene suggests a specific role of FEZ1 in the development of newborn neurons in the adult brain. To further confirm the specificity of the shRNA manipulation, in vivo rescue experiments were performed. We engineered two sets of retroviruses: the first coexpressing GFP and wild-type (WT) mouse *fez1* cDNA without the 3′ untranslated region (3′UTR; pCUXIE-mFEZ1), or GFP alone (pCUXIE); the second coexpressing mCherry and shRNA-F1 (Figure S2A). The shRNA-F1 targets the 3′UTR of the mouse *fez1* gene, thus it does not affect mFEZ1 expression from the rescue vector (pCUXIE-mFEZ1). The two types of engineered retroviruses were coinjected into the adult dentate gyrus (Figure 2A). Expression of shRNA-F1 and mCherry resulted in significant increases in the total dendritic length and soma size in comparison to those expressing shRNA-C1, whereas overexpression of mFEZ1 itself did not lead to any obvious effects (Figures 2B and 2C), except for a modest change in the dendritic complexity, but not the total dendritic length (Figure S2B). Importantly, coexpression of mFEZ1, but not vector control, largely normalized increased

dendritic growth and soma hypertrophy by shRNA-F1 (Figures 2B and 2C). Under all conditions, no significant effects on the number of primary dendrites and neuronal positioning were detected (Figures S2D and S2E). Taken together, these rescue experiments further support specific roles of FEZ1 in regulating distinct aspects of new neuron development in the adult brain.

Synergistic Interaction between FEZ1 and DISC1 in Regulating Development of Newborn Neurons in the Adult Brain

The similar effect of DISC1 and FEZ1 knockdown on dendritic growth and soma size of newborn neurons in the adult brain and reported direct interaction between these two proteins (Miyoshi et al., 2003) suggest that they may functionally interact in regulating neuronal development. We previously generated a collection of shRNAs against mouse *disc1* that exhibit different knockdown efficacy (Duan et al., 2007). Expression of the strong shRNA against *disc1* (shRNA-D1) in newborn neurons led to the full spectrum of phenotypes at 14 dpi, whereas expression of the weak shRNA against *disc1* (shRNA-D3) by itself led to a modest phenotype that manifests at 28 dpi, but not at 14 dpi (Figures 3A and 3C–3E; Figures S3B and S3C) (Duan et al., 2007). To examine a potential interaction between FEZ1 and DISC1, we employed an in vivo double knockdown approach (Figure S3A). Interestingly, coexpression of shRNA-F1 and
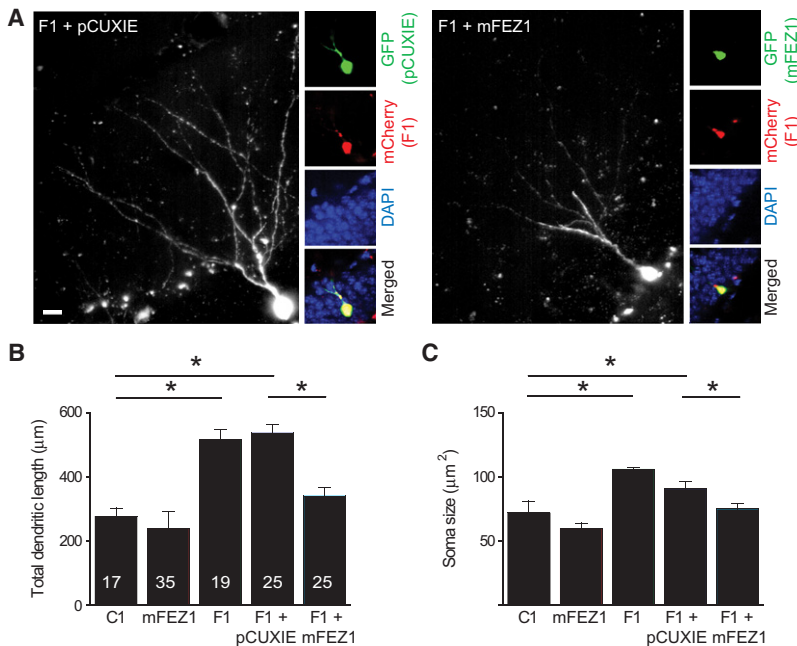
Figure 2. Rescue of Developmental Defects of Newborn Neurons from FEZ1 Knockdown by Overexpression of WT FEZ1

(A) Sample confocal images of double-labeled newborn neurons in the adult dentate gyrus. Retroviruses coexpressing mCherry and shRNA-FEZ1#1 (F1) and those coexpressing GFP and mouse FEZ1 (mFEZ1) or GFP alone (pCUXIE) were coinjected into the adult dentate gyrus and animals were analyzed at 14 dpi after immunostaining for GFP. Shown are sample confocal projection image of GFP staining (left) and images of GFP staining (green), mCherry (red), DAPI (blue), and merged at lower magnification. Scale bar: 10 μm.

(B and C) Summary of total dendritic length (B) and soma size (C) of newborn neuron in the adult brain expressing shRNA-control (C1), mFEZ1, shRNA-FEZ1#1 (F1), or coexpressing F1 and mFEZ1 (F1 + mFEZ1) or control vector (F1 + pCUXIE). Numbers associated with each bar graph refer to the total number of neurons analyzed from at least two animals under each condition. Values represent mean ± SEM (*p < 0.01; ANOVA).

shRNA-D3 exacerbated the dendritic growth phenotype compared with expression of shRNA-F1 alone, as shown in both total dendritic length and complexity (Figures 3B–3D). On the other hand, no apparent synergistic effect was observed for soma size (Figure 3E), number of ectopic dendrites, or positioning of newborn neurons (Figures S3B and S3C). These results suggest that FEZ1 and DISC1 functionally interact to regulate dendritic development of newborn neurons in the adult brain.

To further assess the interaction between endogenous DISC1 and FEZ1 in regulating neuronal development, we explored a blocking peptide using the DISC1 domain that interacts with FEZ1 (aa 446–633 of mouse DISC1; Figure S3D) (Miyoshi et al., 2003). In the coimmunoprecipitation (co-IP) analysis, expression of this peptide in HEK293 cells attenuated interaction between exogenous DISC1 and FEZ1, but not between DISC1 and endogenous KIAA1212/Girdin (Kim et al., 2009) or Kendrin (Shimizu et al., 2008), two other proteins interacting with DISC1 at sites that overlap with this region (Figure S3D). Furthermore, overexpression of this peptide significantly reduced the interaction between endogenous DISC1 and FEZ1 in adult neural progenitors, but had no effect on the interaction between endogenous DISC1 and NDEL1 (Figure S3E), supporting the specificity of the blocking peptide on the DISC1 and FEZ1 interaction. Retrovirus-mediated coexpression of the DISC1 peptide and GFP in newborn neurons in the adult dentate gyrus led to increased total dendritic length and complexity as well as soma hypertrophy of GFP+ neurons at 14 dpi (Figures 3F–3H), but no effect on the number of primary dendrites or neuronal positioning (Figures S3F and S3G), fully recapitulating the FEZ1 knockdown phenotype. Taken together, these results support that FEZ1 regulates specific aspects of new neuron development in the adult brain through functional interaction with DISC1.

Lack of Synergistic Interaction between FEZ1 and NDEL1 in Regulating Development of Newborn Neurons in the Adult Brain

NDEL1 is another DISC1 interacting protein that regulates neuronal development in vivo (Duan et al., 2007; Sasaki et al., 2005; Shu et al., 2004). Consistent with our previous findings (Duan et al., 2007), expression of a specific shRNA against mouse ndel1 (shRNA-N1) led to developmental defects of newborn dentate granule cells, mostly in the appearance of ectopic dendrites and aberrant positioning (Figure 4). Thus, FEZ1 and NDEL1 appear to mediate DISC1 signaling in a complementary set of neuronal developmental processes. To determine whether FEZ1 and NDEL1 also functionally interact to regulate development of newborn neurons, we performed double knockdown experiments in vivo. The effect of coexpressing shRNA-F1 and shRNA-N1 on dendritic growth and soma size of newborn neurons was very similar to those expressing shRNA-F1 alone (Figures 4A–4C), whereas the effect on ectopic dendrites and neuronal positioning was similar to those expressing shRNA-N1 alone (Figures 4D and 4E). Thus, concomitant suppression of NDEL1 and FEZ1 only leads to additive effects of individual knockdown, instead of a synergistic action. These results further support the notion that FEZ1 and NDEL1 differentially regulate distinct aspects of new neuron development in the adult brain.

KIAA1212/Girdin is also a DISC1 binding partner that regulates development of newborn dentate granule cells in the hippocampus (Enomoto et al., 2009; Kim et al., 2009). We next examined whether KIAA1212 interacts with FEZ1 or NDEL1 in regulating neuronal development. Consistent with previous findings, DISC1 was co-IPed with each of the three proteins, NDEL1, FEZ1, or KIAA1212, when each pair was coexpressed in the heterologous system (Figure S4A). Furthermore, these four proteins could be co-IPed together with DISC1 when all were coexpressed (Figure S4A). Also consistent with the previous
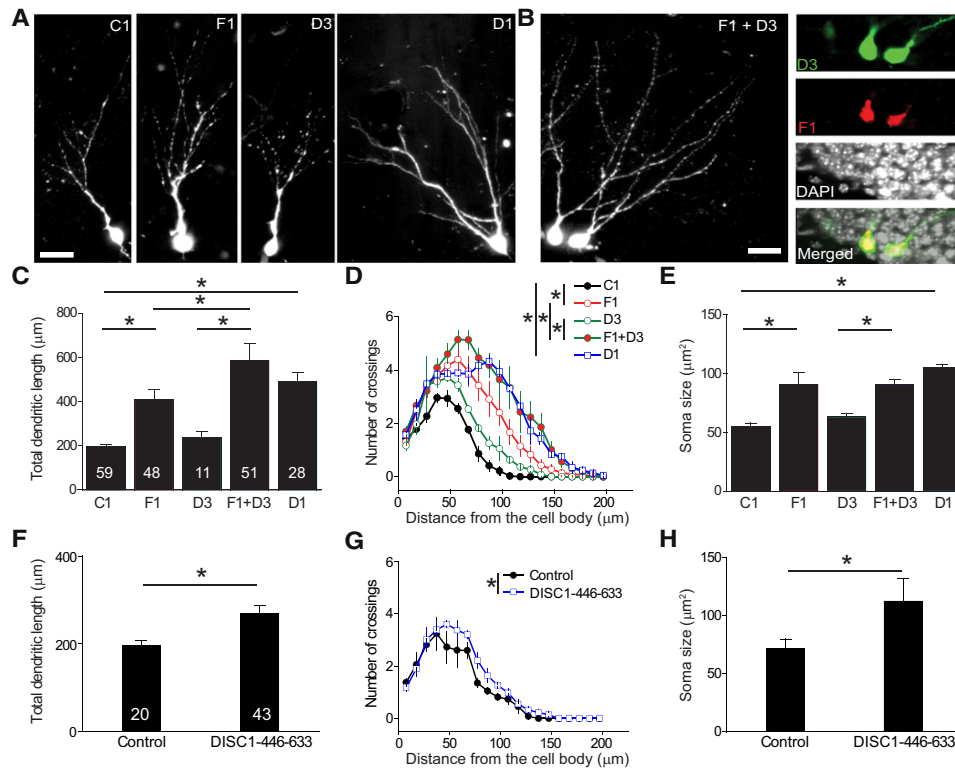
Figure 3. Functional Interaction between FEZ1 and DISC1 in Regulating Development of Newborn Neurons in the Adult Brain

(A–E) Effect of double knockdown of DISC1 and FEZ1 on development of newborn neurons in the adult dentate gyrus. Shown in (A) are sample confocal projection images of GFP^{+} newborn neurons expressing shRNA-control (C1), shRNA-FEZ1#1 (F1), a weak shRNA against mouse *disc1* (shRNA-DISC1#3; D3), or a strong shRNA against mouse *disc1* (shRNA-DISC1#1; D1) at 14 dpi. Shown in (B) are sample confocal images of double-labeled newborn neurons coexpressing shRNA-DISC1#3 (D3)/GFP and shRNA-FEZ1#1 (F1)/mCherry at 14 dpi. Scale bars: 20 μm. Also shown are summary of the total dendritic length (C), Sholl analysis of dendritic complexity (D) and soma size (E) of newborn neuron in the adult brain expressing shRNA-control (C1), shRNA-FEZ1#1 (F1), shRNA-DISC1#3 (D3), or shRNA-DISC1#1 (D1), or coexpressing F1 and D3 (F1 + D3), at 14 dpi. The same group of cells for C1 and F1 as in Figure 1 were plotted for comparison. Values represent mean ± SEM (*p < 0.01; ANOVA).

(F–H) Effect of expression of a DISC1 blocking peptide on development of newborn neurons in the adult dentate gyrus. Retroviruses coexpressing GFP and a peptide encoding a DISC1 domain that interacts with FEZ1 (aa 446–633; Figure S3D) were stereotaxically injected into adult dentate gyrus to infect proliferating progenitors. Shown are summaries of the total dendritic length (F), Sholl analysis (G), and soma size (H) of GFP^{+} neurons at 14 dpi, similarly as in (C–E).

finding (Kim et al., 2009), overexpression of KIAA1212 led to increased total dendritic length, number of primary dendrites, and soma size in newborn neurons in the adult dentate gyrus (Figures S4B–S4D). Compared with KIAA1212 overexpression or FEZ1 knockdown alone, comanipulation exacerbated phenotypes of increased dendritic length and soma size, but not the number of primary dendrites and positioning of newborn neurons (Figures S4B–S4E). On the other hand, simultaneous KIAA1212 overexpression and NDEL1 knockdown exhibited phenotypes very similar to those of NDEL1 knockdown alone (Figures S4B–S4E). Taken together, these results support a model that DISC1 interacts with FEZ1 and KIAA1212 mainly to regulate dendritic growth and soma size of newborn neurons during adult neurogenesis, whereas DISC1 interacts with NDEL1 mainly to regulate positioning of newborn neurons (Table 1).

Biochemical Interaction among FEZ1, DISC1, and NDEL1

To examine the biochemical basis of cooperation among FEZ1, DISC1, and NDEL1 in regulating neuronal development, we examined interactions of endogenous proteins using co-IP analysis of protein lysates from adult mouse neural progenitors in culture. Consistent with previous findings from studies of exogenous proteins (Kamiya et al., 2005; Miyoshi et al., 2003), endogenous DISC1 was co-IPed with FEZ1 and NDEL1, and vice versa (Figure 5A; Figure S5). Furthermore, endogenous NDEL1 was co-IPed with FEZ1, and vice versa (Figure 5A). We obtained similar results with protein lysates from adult mouse hippocampal tissue and with two different anti-DISC1 antibodies (Figures S5A–S5C). These results suggest that FEZ1, DISC1, and NDEL1 comprise a protein complex or complexes in vivo.

To determine whether FEZ1 and NDEL1 interact through the common binding partner DISC1 or independently of DISC1, we performed co-IP experiments using adult mouse neural progenitors expressing shRNA-D1 (Figure 5B). DISC1 knockdown did not affect the endogenous protein expression level of either NDEL1 or FEZ1 in adult neural progenitors (Figure 5B). Interestingly, DISC1 knockdown led to a significant decrease in the co-IP efficacy between FEZ1 and NDEL1 (Figure 5B). In contrast, NDEL1 knockdown did not affect the co-IP efficacy of FEZ1
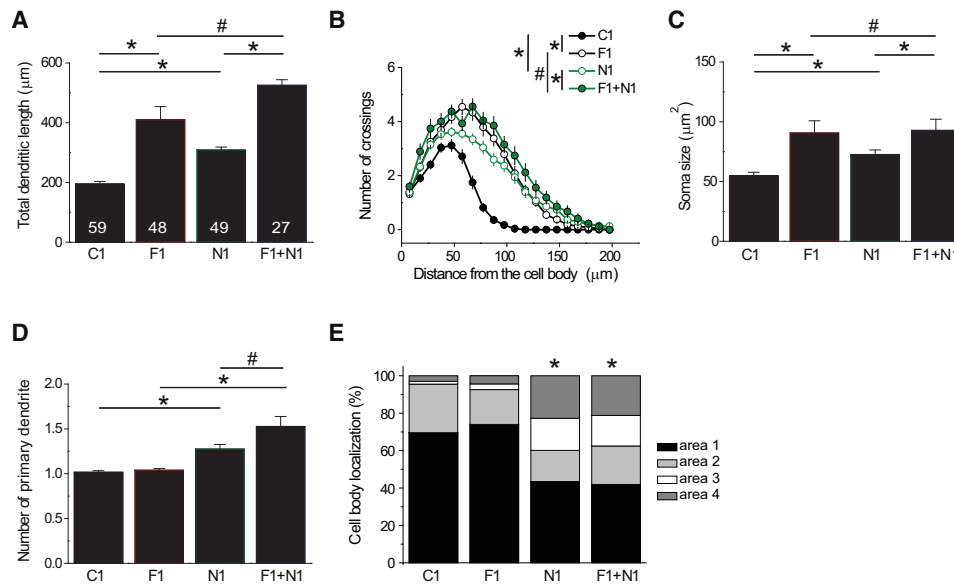
Figure 4. Lack of Functional Interaction between FEZ1 and NDEL1 in Regulating Development of Newborn Neurons in the Adult Brain
Summary of total dendritic length (A), Sholl analysis of dendritic complexity (B), soma size (C), number of primary dendrites (D), and distribution of cell body position within different domains (E, as defined in Figure 1B) of newborn neuron in the adult brain expressing shRNA-control (C1), shRNA-FEZ1#1 (F1), shRNA-NDEL1 (N1), or coexpressing F1 and N1 (F1 + N1), at 14 dpi. The same group of cells for C1 and F1 as in Figure 1 were plotted for comparison. Values represent mean ± SEM (*p < 0.01; #p > 0.05; ANOVA).

and FEZ1 knockdown did not affect the co-IP efficacy of NDEL1 using anti-DISC1 antibodies (Figure 5C). Furthermore, FEZ1 overexpression in HEK293 cells did not appear to hinder the interaction between DISC1 and NDEL1, and vice versa, suggesting a lack of apparent competition between FEZ1 and NDEL1 for binding to DISC1 (Figure S5D).
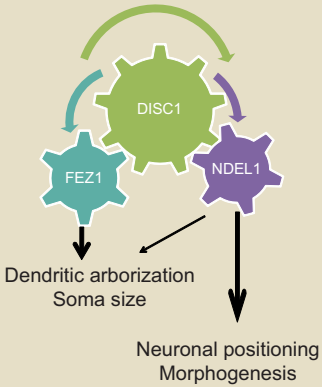
Taken together, these results suggest that DISC1 interacts with both NDEL1 and FEZ1, whereas NDEL1 and FEZ1 appear to form a complex through DISC1, but not directly in vivo. These findings are consistent with our findings of a synergistic interaction between DISC1 and FEZ1 (Figure 3), and between DISC1 and NDEL1 (Duan et al., 2007), but not between FEZ1 and NDEL1 (Figure 4), in regulating distinct aspects of new neuron development in the adult brain (Table 1).

Epistasis between *FEZ1* and *DISC1* for Risk of Schizophrenia

In parallel to examining the FEZ1 role in neuronal development in an animal model, we conducted a genetic association study of *FEZ1* in schizophrenia with a cohort of 279 Caucasian patients with schizophrenia and schizoaffective disorder and 249 Caucasian healthy controls (ZHH cohort) (Burdick et al., 2008). We assessed four SNPs within the *FEZ1* gene, spanning B36 positions 124834271 to 124858699 (rs12224788; rs10893385; rs618900; rs2849222) (Figure 6A). The linkage disequilibrium (LD) among the four SNPs comprising the haplotypes was high with D-prime values of 0.93 or greater. All SNPs were in Hardy-Weinberg equilibrium (HWE; data not shown). However, χ^2 analyses revealed that none of the four genotyped SNPs were associated with a significant risk for schizophrenia (Table S1A). In addition,

there were no significant haplotype associations with schizophrenia susceptibility (Table S1A).

Several converging lines of evidence have suggested that *DISC1* Ser704Cys is of particular importance in increasing risk for schizophrenia through modifying DISC1 protein interactions (Burdick et al., 2008; Callicott et al., 2005; DeRosse et al., 2007; Lipska et al., 2006). We thus examined potential epistatic interactions between each of the four *FEZ1* SNPs and the *DISC1* Ser704Cys locus. Owing to the low frequency of the Cys allele, *DISC1* Ser704Cys genotypes were grouped to compare subjects carrying at least one copy of the Cys allele (Cys) with subjects homozygous for the Ser allele (SerSer). For all *FEZ1* SNPs, we grouped minor allele carriers and major allele homozygotes to optimize power based on genotype frequencies (Table S1B). We first investigated the possible influence that an interaction between *FEZ1* and *DISC1* might have on risk for schizophrenia by carrying out four separate χ^2 analyses with one for each *FEZ1* SNP, while conditioning the sample on *DISC1* Ser704Cys status. These analyses revealed that the C allele at *FEZ1* rs12224788 increased risk for schizophrenia in the context of a *DISC1* SerSer background (χ^2 = 4.75; df = 1; p = 0.029; odds ratio [OR] = 2.55; 95% confidence interval [CI]: 1.1–6.0; Fisher's exact p value = 0.046), but was not significant in patients carrying 1 or 2 copies of the Cys allele (χ^2 = 0.18; df = 1; p = 0.67; OR = 0.82; 95% CI: 0.3–2.1; Fisher's exact p value = 0.815; Figure 6B). Likewise, *FEZ1* rs10893385 χ^2 results indicated a potential interaction with *DISC1* Ser704Cys such that T allele carriers were at significantly increased risk for schizophrenia in the background of *DISC1* SerSer (χ^2 = 3.84; df = 1; p = 0.050; OR = 0.49; 95% CI: 0.2–1.0), but not in *DISC1* Cys

Table 1. A Model of Interaction between DISC1 with FEZ1 or NDEL1 in Regulating Distinct Aspects of Newborn Neuron Development in the Adult Mouse Hippocampus

shRNA	Neuronal positioning	Soma hypertrophy	Ectopic dendrites	Enhanced dendritic outgrowth
Control	o	o	o	o
DISC1#1	+++	+++	+++	+++
FEZ1	o	+++	o	++
NDEL1	++	+	++	+
DISC1#3	o	o	o	o
FEZ1+DISC1#3	o	+++	o	++++
FEZ1+NDEL1	++	+++	++	+++
DISC1#3+NDEL1	++	+++	+++	+++

Shown on the left is a schematic diagram of the model on signaling mechanisms of DISC1 in regulating new neuron development during adult hippocampal neurogenesis. Shown on the right is a summary table of effects of different genetic manipulations that lead to defects in neuronal positioning, soma hypertrophy, ectopic dendrites, and enhanced dendritic outgrowth of newborn neurons in the adult dentate gyrus at 14 dpi ("o" represents normal and the severity of phenotypes is represented by the numbers of "+").

Carriers ($\chi^2 = 0.14$; df = 1; p = 0.711; OR = 1.16; 95% CI: 0.5–2.5) (Figure S6A). Neither *FEZ1* rs618900 nor rs2849222 showed any evidence of interaction at the χ^2 level (Figures S6B and S6C). Therefore, to test for statistical evidence of a true epistatic interaction, we used the likelihood ratio test in a backward stepwise regression and included *DISC1* Ser704Cys genotype, *FEZ1* rs12224788 genotype, *FEZ1* rs10893385, and an interaction term for each *FEZ1* SNP (*DISC1* × *FEZ1*). While none of the main effects for genotype were significant [*DISC1* Ser704Cys (Beta = −0.27, p = 0.69); *FEZ1* rs12224788 (Beta = −0.18, p = 0.701); *FEZ1* rs10893385 (Beta = −0.08, p = 0.888)], the *FEZ1* rs12224788 × *DISC1* Ser704Cys interaction term was significant (Beta = 0.54, p = 0.028), indicating evidence of epistasis (Figure 6C). Despite the χ^2 results, the interaction term for *FEZ1* rs10893385 × *DISC1* Ser704Cys was not significant (Beta = −0.42, p = 0.211), suggesting that this is not an epistatic relationship. The final model classified subject type with 59% accuracy with a $\chi^2 = 4.88$ (p = 0.027).

Subsequent analyses were conducted in the much larger Genetic Association Information Network (GAIN) sample set to test for a replication of our ZHH results. The GAIN sample consisted of 1351 schizophrenia cases (29.9% female; mean age 43.3 ± 11.4 years) and 1378 healthy controls (54.0% female; mean age 51.1 ± 17.0 years) for which genotype data at the four *FEZ1* SNPs were available (Table S1A). No significant results were detected on susceptibility to schizophrenia for each of the four *FEZ1* SNPs, which is consistent with the result from the ZHH cohort (Table S1A). The platform used to genotype GAIN (Affymetrix 6.0 chip) did not include the *DISC1* Ser704Cys marker but has a perfect proxy for this SNP (rs1754605; r2 = 1.0). We then performed a backward stepwise regression to test for an interaction between the proxy SNP for *DISC1* Ser704Cys and *FEZ1* rs12224788 (Figure 6C). As this approach was to serve as a replication of the findings in the ZHH data set, we included only the *FEZ1* SNP with statistical evidence of epistasis (*FEZ1*

rs12224788) in the GAIN sample regression model (Table S1B). Variables retained in the best fit model included *FEZ1* genotype (Beta = 0.72; p = 0.041), *DISC1* genotype (Beta = 0.53; p = 0.044), and the interaction term *FEZ1* × *DISC1* (Beta = −0.45; p = 0.039). While the significant interaction is consistent with results from the ZHH data set, the Beta term is negative, suggesting a somewhat different pattern of interaction in the GAIN sample as compared with the ZHH sample. Specifically, χ^2 analyses revealed only a trend-level association for the C allele at *FEZ1* rs12224788 in *DISC1* Ser homozygotes ($\chi^2 = 1.53$; df = 1; p = 0.12; OR = 1.2) and a significant association for the *FEZ1* GG genotype in the context of a *DISC1* Cys background ($\chi^2 = 2.83$; df = 1; p = 0.05; OR = 0.77; Figure 6B). While not identical, this pattern of risk association related to the interaction is consistent with that found in the ZHH sample.

We also performed a series of χ^2 tests in the same way to test for a potential interaction between our *NDEL1* risk SNP (rs1391768) (Burdick et al., 2008) and each of the four *FEZ1* SNPs using the ZHH sample. We carried out four separate χ^2 analyses with one for each *FEZ1* SNP, while conditioning the sample on *NDEL1* rs1391768 status. The results from these analyses provided no significant evidence of interaction among these four *FEZ1* SNPs and *NDEL1* rs1391768 (all p > 0.10; Figure S6D and data not shown).

Taken together, these genetic interaction results from clinical cohorts mirror the biochemical and cell biological findings of a synergistic interaction between FEZ1 and DISC1, but not between FEZ1 and NDEL1, in regulating neuronal development in the animal model.

DISCUSSION

Cumulative evidence supports a significant neurodevelopmental contribution to the pathophysiology of schizophrenia and other major mental disorders (Lewis and Levitt, 2002; Rapoport
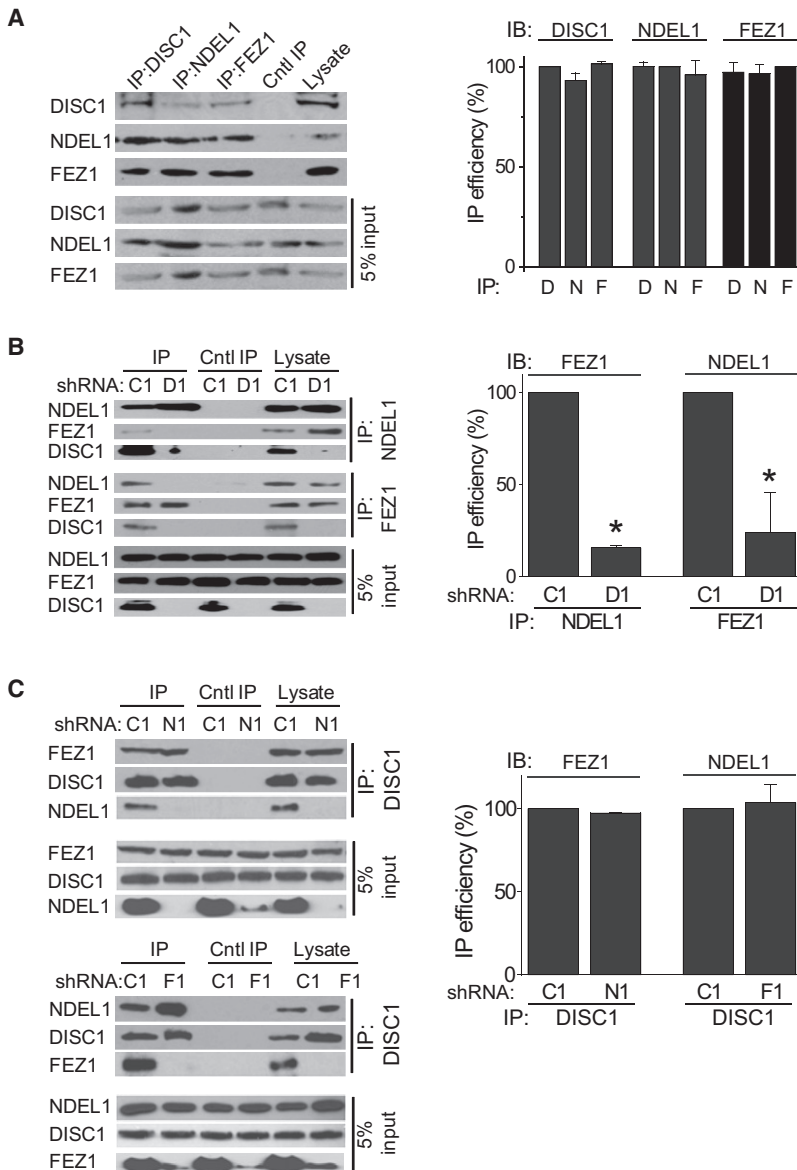
Figure 5. Biochemical Interaction among FEZ1, DISC1, and NDEL1

(A) Association of endogenous DISC1, FEZ1, and NDEL1. Cultured adult mouse neural progenitors were subjected to co-IP analysis using antibodies against DISC1, NDEL1, or FEZ1, respectively. A summary of quantification of co-IP efficacy is also shown. Values represent mean ± SEM (n = 3; p > 0.05; ANOVA).

(B) Lack of interaction between FEZ1 and NDEL1 in adult neural progenitors with DISC1 knockdown. Adult neural progenitors were infected with retroviruses to express shRNA-DISC1 (D1) or shRNA-control (C1). After 48 hr, cell lysates were subjected to western blot analysis for expression of DISC1, FEZ1, or NDEL1, or subjected to co-IP analysis using antibodies against FEZ1 or NDEL1, respectively. A summary of quantification of co-IP efficacy is also shown. Values represent mean ± SEM (n = 3; *p < 0.01; ANOVA).

(C) Independent interaction between DISC1 and FEZ1 and between DISC1 and NDEL1. Adult neural progenitors were infected with retroviruses to express shRNA-NDEL1 (N1), shRNA-FEZ1 (F1), or shRNA-control (C1). After 48 hr, cell lysates were subjected to co-IP analysis using antibodies against DISC1. A summary of quantification of co-IP efficacy is also shown. Values represent mean ± SEM (n = 3; p > 0.05; ANOVA).

as an "edge piece" of psychiatric genetics, DISC1 may thus provide an entry point to understand molecular mechanisms and etiology underlying complex psychiatric disorders. Using a combinatorial approach to analyze the effect of genetic manipulations on individual neurons in the animal model, biochemical interactions of endogenous proteins in a homogenous cell population, and genetic associations in clinical cohorts, we demonstrate two parallel pathways for FEZ1 and NDEL1 that independently cooperate with DISC1 to regulate different aspects of neuronal development and risk for schizophrenia.

In the dentate gyrus of the hippocampus, a region implicated in schizophrenia pathophysiology (Harrison, 2004), neurogenesis continues throughout life in all mammals and contributes to specific brain functions (Zhao et al., 2008). Adult hippocampal neurogenesis provides a unique model system for dissecting signaling mechanisms that regulate neurodevelopment and offers several distinct advantages for molecular analysis, including a prolonged developmental time course for more precise temporal resolution, a single neuronal subtype, and amenability to birth-dating, lineage tracing, and genetic manipulations (Christian et al., 2010). Using this in vivo model system, we have identified novel functions of FEZ1 in regulating dendritic growth and soma size of newborn dentate granule cells in the adult hippocampus (Figure 1). Furthermore, results from concomitant suppression of DISC1 and FEZ1 support a synergistic interaction between these two proteins in regulating dendritic growth in vivo (Figure 3). In parallel, the NDEL1-DISC1 interaction regulates a complementary subset

et al., 2005; Weinberger, 1987), yet underlying molecular mechanisms are far from clear. DISC1 has emerged as a general risk factor for schizophrenia, schizoaffective disorder, bipolar disorder, major depression, autism, and Asperger syndrome (Chubb et al., 2008; Muir et al., 2008). In support of a role of DISC1 in mental disorders, dysfunction of DISC1 in rodent models leads to several schizophrenia and/or depression-related behavioral phenotypes (Johnstone et al., 2011). At the cellular level, expression of DISC1 is developmentally regulated within the nervous system (Miyoshi et al., 2003) and DISC1 in turn regulates multiple processes of both embryonic and adult neurogenesis (Christian et al., 2010). At the molecular level, a large number of potential DISC1 binding partners have been identified from a yeast two-hybrid screen (Chubb et al., 2008), many of which are also involved in neurodevelopmental processes implicated in the pathophysiology of psychiatric diseases. Regarded
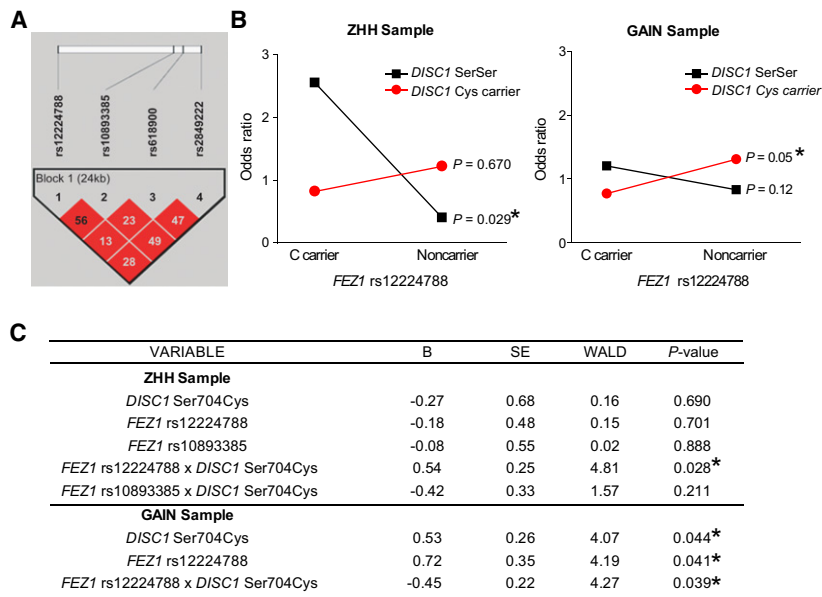
Figure 6. Epistatic Interaction between *FEZ1* and *DISC1* for the Risk of Schizophrenia

(A) Linkage disequilibrium (χ^2) structure of *FEZ1* haplotype block using Haploview 3.32. The four SNPs formed a single haplotype block within the *FEZ1* gene.

(B) A summary plot of χ^2 analyses with Zucker Hillside Hospital (ZHH) samples (left) and the Genetic Association Information Network (GAIN) samples (right) to depict the *DISC1-FEZ1* genotype interaction graphically. Subjects are grouped by genotype at both *FEZ1* rs12224788 and *DISC1* Ser704Cys. The x and y axes represent *FEZ1* genotype group and odds ratio respectively. The likelihood ratio test from the regression analysis indicated a significant *FEZ1* × *DISC1* interaction (p = 0.029 for ZHH sample and p = 0.05 for the GAIN sample).

(C) Summary of the likelihood ratio test in a backward stepwise regression with ZHH and GAIN samples (See Table S1B for sample size and composition). Genotypes are dichotomized for regression analyses. The same sets of samples as in (B) were analyzed. (*p < 0.05).

VARIABLE	B	SE	WALD	*P*-value
ZHH Sample				
DISC1 Ser704Cys	-0.27	0.68	0.16	0.690
FEZ1 rs12224788	-0.18	0.48	0.15	0.701
FEZ1 rs10893385	-0.08	0.55	0.02	0.888
FEZ1 rs12224788 x *DISC1* Ser704Cys	0.54	0.25	4.81	0.028*
FEZ1 rs10893385 x *DISC1* Ser704Cys	-0.42	0.33	1.57	0.211
GAIN Sample				
DISC1 Ser704Cys	0.53	0.26	4.07	0.044*
FEZ1 rs12224788	0.72	0.35	4.19	0.041*
FEZ1 rs12224788 x *DISC1* Ser704Cys	-0.45	0.22	4.27	0.039*

of developmental processes, namely, neuronal positioning and development of primary dendrites (Duan et al., 2007). Interestingly, there is no apparent synergistic interaction between FEZ1 and NDEL1 in regulating neuronal development (Figure 4) and no protein-protein interaction in the absence of DISC1 (Figure 5). These results illustrate two discrete pathways associated with the DISC1 interactome that, in conjunction, account for most of the DISC1-mediated effects in orchestrating development of newborn neurons during adult hippocampal neurogenesis (Table 1). Despite a large number of potential DISC1 interactors and multiple functions of DISC1 at different developmental stages and in different brain regions, our study demonstrates the feasibility of dissecting complex signaling pathways to identify molecules that mediate each of these specific aspects of DISC1 function in vivo.

Schizophrenia is a heterogeneous disease with complex genetic contributions. There are at least two non-mutually exclusive models to explain how genetic variations contribute to the risk for schizophrenia. In the "common disease - common alleles" model, an increased risk of schizophrenia stems from combined effects of multiple common polymorphisms that incrementally impact the overall susceptibility (Chakravarti, 1999). In the "common disease - rare alleles" model, schizophrenia is a common disease precipitated by the presence of rare alleles that individually confer significant risk with high penetrance (McClellan et al., 2007). In the case of DISC1, the chromosome translocation that disrupted *DISC1* in the original Scottish family increased the risk of developing schizophrenia and other major mental disorders by about 50-fold compared with the general population (Blackwood et al., 2001), supporting the model of "common disease - rare alleles." So far, genome-wide association studies (GWAS) of schizophrenia, including a recent large meta-analysis (Mathieson et al., 2011), have not yet shown a significant association with the *DISC1* locus. Association of DISC1 haplotypes with schizophrenia and other mental illness

has been found in some populations, but not others (Chubb et al., 2008). For example, one *DISC1* SNP on exon 11 (rs821616, Ser704) has been identified as a risk allele (Callicott et al., 2005) and associated with positive symptoms in schizophrenia only in some populations (DeRosse et al., 2007). A number of studies identified other genes, including *FEZ1*, which indicate susceptibility in some populations, but cannot be confirmed in others. The failures to replicate risk association of specific genes might reflect small marginal effects, while the possibility of interaction is often overlooked due to computational and statistical limitations in the absence of preexisting hypotheses of specific gene pairs. In fact, epistatic interactions have been suggested as a major component of the "missing heritability" witnessed by GWAS (Eichler et al., 2010). Our analysis of a cohort of 279 patients with schizophrenia and 249 healthy controls suggests a lack of significant direct association of variation within the *FEZ1* gene and risk for schizophrenia. Instead, we found an epistatic interaction between *FEZ1* rs12224788 and *DISC1* Ser704Cys, which significantly influences schizophrenia susceptibility. Specifically, an approximate 2.5-fold increased risk for schizophrenia is seen in individuals carrying the C allele at *FEZ1* rs12224788, but only in the context of a *DISC1* Ser704Ser background with no significant effect in *DISC1* Cys carriers. Importantly, our analysis of a second cohort from the GAIN date set with independent and larger number of samples supports a similar conclusion (Figure 6). Whether our statistical evidence of epistasis reflects disruption of molecular interactions between DISC1 and FEZ1 involving coding variants in linkage disequilibrium with rs12224788, or whether rs12224788 tags a regulatory variant, remains unclear and is an interesting lead for future studies. Interestingly, there is also an epistatic interaction between *NDEL1* rs1391768 and *DISC1* Ser704Cys only in the context of a *DISC1* Ser704Ser background (Burdick et al., 2008). On the other hand, we did not find any significant epistatic interaction between the four *FEZ1*

SNPs and four *NDEL1* SNPs (Figure S6 and data not shown), although we cannot rule out the possibility of an epistatic interaction of these two genes at other SNPs. In a recent study of epistasis based on machine learning algorithms and functional magnetic resonance imaging (fMRI) analysis, significant interaction was found between *DISC1*, *CIT*, and *NDEL1* SNPs (Nicodemus et al., 2010). Taken together, these findings put DISC1 at the center of a signaling complex in which its interaction with different partners confers increased risk for schizophrenia as well as regulating different aspects of neuronal development.

Our results may begin to reconcile the contrasting views on genetically determined disease susceptibility. DISC1 is a multivariate modulator of risk conference with high penetrability and represents an essential component of divergent pathways that regulate disease and development. Due to this partial functional overlap between DISC1 and its binding partners, DISC1 emerges as a key player in disease susceptibility, whereas genes regulating a subset of DISC1 functions may only incrementally increase overall risk. Our results thus demonstrate how the two prevailing views of genetically conferred disease susceptibility are compatible in mechanistic terms. We provide evidence in support of large effects from the disruption of a single gene (*DISC1*) and how polymorphisms in *DISC1* and associated genes can work synergistically, through epistatic mechanisms, resulting in increased risk for schizophrenia. Importantly, this synergistic interaction also reveals how genetic context is critical in determining the extent of susceptibility to disease pathogenesis. As shown in our association results, an individual SNP confers differential risk effects depending on the genetic background of the patient. Because of the prohibitively large number of genes that have been identified as potential risk factors for schizophrenia and related disorders, an efficient method to determine the relevant genetic interactions is through biochemical and cellular assays based on functional analysis. We provide an example of how a targeted investigation of molecular pathways associated with DISC1 functions can generate testable hypotheses of genetic interactions in the patient population. By continuing to investigate functional interactions of key genes associated with disease susceptibility at the cellular level, we can begin to unravel the complex genetic contributions to neuropsychiatric disorders.

EXPERIMENTAL PROCEDURES

Constructs, Cell Culture, and Biochemistry

Engineered self-inactivating murine oncoretroviruses were used to coexpress shRNAs under the U6 promoter and GFP or mCherry under the EF1α promoter (pUEG/pUEM vector), or to coexpress mouse *fez1* cDNA (without the 3′UTR) under the Ubiquitin C promoter and GFP following the IRES sequence (pCUXIE vector), specifically in proliferating cells and their progeny in vivo (Duan et al., 2007). shRNAs against mouse *disc1* (shRNA-D1, shRNA-D3) and *ndel1* (shRNA-N1) have been previously characterized (Duan et al., 2007; Faulkner et al., 2008). Two *fez1* shRNAs were designed to target the 3′UTR of mouse *fez1* gene with following sequences: shRNA-FEZ1#1 (F1): 5′-CTTATACTCTTAAGACTAA-3′; shRNA-FEZ1#2 (F2): 5′-GCGTGTATTTAAACGTGTA-3′. The control shRNA vector (shRNA-C1; C1) contains a scrambled sequence without homology to any known mammalian mRNA: 5′-TTCTCCGAACGTGTCACGT-3′ (QIAGEN).

Neural progenitors were isolated from adult mice hippocampi (C57BL/6) and cultured as a monolayer as previously described (Kim et al., 2009;

Ma et al., 2008). At 48 hr after retroviral infection, cell lysates were prepared in the lysis buffer containing 10% glycerol, 1% nonylphenoxypolyethoxy ethanol (Nonidet P-40), 50 mM Tris-Cl (pH 7.5), 200 mM NaCl, 2 mM $MgCl_2$, 0.2 mM Na_3VO_4, and 1 μg/ml protease inhibitor cocktail (Roche). Protein lysates were subjected to western blot analysis for FEZ1 (goat, 1:1000; Novus), DISC1 (goat, 1:1000; Santa Cruz), NDEL1 (rat, 1:1000; gift of A. Sawa) (Kamiya et al., 2005), and GAPDH (mouse, 1:1000; Abcam). For co-IP analysis, both adult mouse neural progenitors at 48 hr after retroviral infection and dissected hippocampal tissues from adult mice were used as previously described (Kim et al., 2009). Samples were immunoprecipitated with antibodies against DISC1 (goat, 1:100; Santa Cruz, or rabbit, 1:100; Zymed), FEZ1, or NDEL1, and then subjected to western blot analysis. Blots were stripped and reblotted with the same antibodies used for their immunoprecipitation to ensure equal loading. For quantification, the densitometry measurement of each band (Image J) was first normalized to that of GAPDH and then averaged from at least three independent experiments.

In Vivo Genetic Manipulation of Neural Progenitors with Engineered Retroviruses

High titers of engineered retroviruses were produced as previously described (Duan et al., 2007). Adult female C57BL/6 mice (7–8 weeks old; Charles River) housed under standard conditions were anaesthetized. Concentrated retroviruses were stereotaxically injected into the dentate gyrus at four sites (0.5 μl per site at 0.25 μl/min) with the following coordinates (in mm; posterior = 2 from Bregma, lateral = ± 1.6, ventral = 2.5; posterior = 3 from Bregma, lateral = ± 2.6, ventral = 3.2) as previously described (Duan et al., 2007). All animal procedures used in this study were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee.

Immunohistology, Confocal Imaging, and Analysis

Coronal brain sections (40 μm thick) were prepared from injected mice at 14 dpi and processed for immunostaining as previously described (Duan et al., 2007; Ge et al., 2006). For rescue experiments (Figure 2), anti-GFP antibodies (rabbit, 1:1000, Abcam) were used to enhance the signaling for all conditions in parallel. The sections were incubated for 30 min in 4′,6′-diaminodino-2-phenylindole (DAPI, 1:5000) before washing and mounting. Images were acquired on a META multiphoton confocal system (Zeiss LSM 510) using a multi-track configuration. At least two animals for each condition were analyzed. Statistical comparison of data was performed by ANOVA.

For analysis of cell morphology, Z-series stacks of confocal images were taken and a single confocal image slice with the largest soma area for individual GFP$^+$ neurons was used for quantification using NIH ImageJ program. For analysis of neuronal positioning, single section confocal images of GFP$^+$ neurons with DAPI staining were used to determine the cell localization within four areas as defined in Figure 1B. For analysis of dendritic development, three-dimensional (3D) reconstruction of entire dendritic processes of each GFP$^+$ neuron was made from Z-series stacks of confocal images. The 2D projection images were traced with NIH ImageJ. All GFP$^+$ dentate granule cells with largely intact dendritic trees were analyzed for total dendritic length as previously described (Duan et al., 2007; Ge et al., 2006). The measurements did not include corrections for inclinations of dendritic process and therefore represented projected lengths. Sholl analysis for dendritic complexity was carried out by counting the number of dendrites that crossed a series of concentric circles at 10 μm intervals from the cell soma as previously described (Duan et al., 2007). For analysis of axonal development, cross-sections (50 μm thick) taken perpendicular to the long axis (septaltemporal) were prepared from dissected hippocampi of fixed mouse brain at 14 dpi and processed for immunostaining using anti-GFP antibodies (goat, 1:500, Rockland) and DAPI (1:5000). 3D reconstructions of entire images were obtained using stitch and maximum intensity projection functions in the Zen software (Zeiss).

Analysis of Cohorts of Schizophrenia Patients and Healthy Controls

For the ZHH sample, the same study group as previously analyzed for *DISC1* and *NDEL1* interaction were used (Burdick et al., 2008). Briefly, the cohort included 279 Caucasian patients with schizophrenia or schizoaffective

disorder (SZ; 35.5% female) with a mean age of 38.6 ± 10.8 years and an estimated IQ of 96.6 ± 8.5 (based on WRAT-3 Reading). All subjects provided written informed consent to an Institutional Review Board of the North Shore-Long Island Jewish Health System (NSLIJHS)-approved protocol. Clinical characteristics that were collected included duration of illness (17.0 ± 10.8 years) and global assessment of function (GAF; 38.7 ± 15.6). Caucasian healthy control subjects (HC; n = 249) were recruited from the general population. Subjects were excluded if they had an Axis I diagnosis, active or recent substance abuse, or if they had a first-degree relative with a known or suspected Axis I disorder based on family history questionnaire. Controls were 52.2% female, had a mean age of 48.4 ± 18.6 years and an estimated IQ of 105.0 ± 12.4. All subjects were Caucasian by self-report and drawn from a single geographic location (Glen Oaks, NY area). Although population stratification is a potential confound in any case-control study, we have previously demonstrated that undetected substructure is not present in our geographically homogeneous population. In a genome-wide association study of a case-control cohort collected by the same methods described above (Lencz et al., 2007), we tested for stratification using 210 ancestry informative markers selected for maximal informativeness and observed no differences between patients and controls beyond chance levels. Moreover, none of the subjects in the cohort deviated from a single population as assessed by the STRUCTURE program (Pritchard et al., 2000). Patient diagnosis was established through structured interview (Structured Clinical Interview-DSM-IV; SCID-IV) (First et al., 1998) and confirmed by diagnostic consensus conference, which utilizes expert clinical opinion alongside SCID-IV data and corroborating medical record information. Healthy controls for the project were assessed using the Structured Clinical Interview for DSM-IV, Non-Patient edition, specifically designed for healthy subjects to rule out Axis I diagnoses. In addition to the structured diagnostic interview, potential subjects were screened to rule out any history of CNS trauma, neurological disorder, or diagnosed learning disability.

FEZ1 genotyping procedures were carried out using the Affymatrix 500K platform as previously described (Lencz et al., 2007). Briefly, genomic DNA was extracted from whole blood and hybridized to two chips containing ~262,000 and ~238,000 SNPs based on manufacturer's specifications. Patients and controls were proportionally distributed on each 96-well plate. Genotype calls were made using Bayesian Robust Linear Model with Mahalanobis distance classifier algorithm threshold at 0.5 applied to batches of 100 samples. Mean call rates <90% on both chips, or <85% on one chip, were rejected, resulting in a mean call rate for the retained sample of 97%. Allele frequencies, Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium structure were examined using Haploview 3.32 (Barrett et al., 2005). The four SNPs formed a single haplotype block as shown in Figure 6A.

For *FEZ1* association with illness, none of the four SNPs in the haplotype deviated from HWE (p > 0.05). χ^2 test statistics were used to test for allelic and genotypic associations to schizophrenia for single SNPs, as well as for group differences on haplotype frequencies. For *FEZ1* and *DISC1* epistasis, we tested for a genotype interaction between each of the four genotyped *FEZ1* SNPs and *DISC1* Ser704Cys by utilizing a likelihood ratio test in an unconditional logistic regression model, with subject type (SZ versus HC) as the dependent measure and entering *FEZ1* rs12224788 genotype, *DISC1* Ser704Cys genotype, and the interaction term *FEZ1* × *DISC1* in a backward stepwise model. In addition, χ^2 analyses were conducted to determine odds ratios for the significant interaction, by conditioning the sample on DISC1 Ser704Cys. Identical statistical methods were utilized when investigating the potential interactions between *FEZ1* and *NDEL1*, focusing on our previously identified *NDEL1* risk SNP (rs1391768) (Burdick et al., 2008) and testing each of the four *FEZ1* SNPS for interaction.

The Molecular Genetics of Schizophrenia (MGS) sample from the Genetic Association Information Network (GAIN) included 1351 Caucasian schizophrenia cases and 1378 healthy controls with available genotype data at the four *FEZ1* SNPs. The platform used to genotype the GAIN samples was the Affymetrix 6.0 array (Shi et al., 2009). The schizophrenia sample was 29.9% female (mean age: 43.3 ± 11.4 years). The GAIN controls were 54.0% female (mean age: 51.1 ± 17.0 years). Analyses were carried out using the identical methodology as those used for the ZHH sample. First, χ^2 analyses were con-

ducted to test for association of the four *FEZ1* SNPs with risk for schizophrenia. Next, we carried out a backward stepwise regression to test for an interaction between the proxy SNP for *DISC1* Ser704Cys and *FEZ1* rs12224788. Only the *FEZ1* SNP with statistical evidence of epistasis (*FEZ1* rs12224788) in the ZHH analyses was included in the GAIN sample regression model, as this was meant to serve as a replication cohort.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and one table and can be found with this article online at doi:10.1016/j.neuron.2011.09.032.

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