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# *Functional genomics in postmortem human brain: abnormalities in a* DISC1 *molecular pathway in schizophrenia*

*Barbara K. Lipska, PhD; Shruti N. Mitkus, PhD; Shiny V. Mathew, PhD; Robert Fatula; Thomas M. Hyde, MD, PhD; Daniel R. Weinberger, MD; Joel E. Kleinman, MD, PhD*

*The disrupted in schizophrenia 1 (*DISC1*) gene has been identified as a schizophrenia susceptibility gene based on linkage and single nucleotide polymorphism (SNP) association studies and clinical data, suggesting that risk SNPs impact on hippocampal structure and function. We hypothesized that altered expression of* DISC1 *and/or its molecular partners (nuclear distribution element-like* [NUDEL]*, fasciculation and elongation protein zeta-1* [FEZ1]*, and lissencephaly 1* [LIS1]*) may underlie its pathogenic role in schizophrenia and explain its genetic association. We examined the expression of* DISC1 *and its binding partners in the hippocampus and dorsolateral prefrontal cortex of postmortem human brains of schizophrenic patients and controls. We found no difference in the expression of* DISC1 *mRNA in schizophrenia, and no association with previously identified risk SNPs. However, the expression of* NUDEL*,* FEZ1*, and* LIS1 *was significantly reduced in tissue from schizophrenic subjects, and the expression of each showed association with high-risk* DISC1 *polymorphisms. These data suggest involvement of genetically linked abnormalities in the* DISC1 *molecular pathway in the pathophysiology of schizophrenia.*

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Schizophrenia is a syndrome characterized by psychotic symptoms (hallucinations, delusions, thought disorder, and cognitive impairment), with a prevalence approaching 1% worldwide. Schizophrenia is clearly a genetic disorder. Results from twin and adoption studies show a heritability estimate for schizophrenia of 70% to 90%.[1-3] However, analysis of recurrence risk estimates in families with one or more affected individuals clearly argues against schizophrenia being a single-gene disorder, even with the possibility of incomplete penetrance.[4] As in other psychiatric disorders, the mode of transmission for schizophrenia is complex and multifactorial, with the possibility of a number of genes conferring varying degrees of susceptibility. With this in mind, efforts have been directed at identifying allelic variants in genes that may confer increased risk for schizophrenia. Identification of schizophrenia susceptibility genes will also increase our understanding of the molecular pathways involved in the etiology of the disorder, and may offer new therapeutic targets.

## DISC1 gene

The disrupted in schizophrenia 1 (*DISC1*) gene is a 414.3 kb gene located on chromosomal region 1q42.2, and consists of 13 exons. *DISC1* was originally identified as a candidate gene for schizophrenia in a large Scottish family, in

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**Author affiliations:** Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Md, USA

**Address for correspondence:** 10 Center Drive, Bldg 10, Rm 4N306, Bethesda, MD 20892-1385, USA
(e-mail:lipskab@intra.nimh.nih.gov)

which a balanced translocation involving chromosomes 1 and 11 was strongly linked to schizophrenia, schizoaffective disorder, bipolar affective disorder, and recurrent major depression.[5] In this family, carriers of the translocation were found to have reduced P300 amplitude, which is observed in some patients with schizophrenia.[6] Subsequent association studies identified numerous polymorphisms in the *DISC1* gene associated with schizophrenia and affective disorders, although different polymorphisms/haplotypes in various regions of the gene were implicated in these studies.[7-12]

In the adult mouse brain, *DISC1* is expressed widely, including in the olfactory bulb, cortex, hippocampus, hypothalamus, cerebellum, and brain stem. During development, DISC1 protein is detected at all stages, from embryonic day 10 (E10) to 6 months old, with two significant peaks of protein expression of one of the *DISC1* isoforms at E13.5 and postnatal day 35.[13] Interestingly, these time points correspond to periods of active neurogenesis and puberty in the mouse. These results suggest that *DISC1* may play a critical role in brain development, lending support to the neurodevelopmental hypothesis of schizophrenia.

*DISC1* encodes an 854-amino acid (aa) protein, which shows no homology to other known proteins and little homology between species.[14-16] This amino-acid sequence predicts that the protein DISC1 may act as a scaffolding protein with multiple binding motifs, facilitating formation of protein complexes. The N-terminus (aa 1-347) contains nuclear localization signals, whereas the C-terminus (aa 348-854) appears to be important for microtubule and centrosomal targeting,[17-19] although no centrosomal localization has been detected so far for the native protein.

Although the precise function of DISC1 in the brain is unknown, a number of DISC1-interacting partners have been identified, including fasciculation and elongation protein zeta-1 (FEZ1), nuclear distribution element-like (NUDEL), and lissencephaly 1 (LIS1), which are known to play a role in neuronal development and functioning. Altered interactions between DISC1 and its binding partners are currently being investigated in order to understand more accurately the biology of DISC1 as a schizophrenia susceptibility gene.

## DISC1 molecular pathway

In an effort to understand the cellular function of DISC1, yeast-two hybrid studies have been used to identify molecular interactors of DISC1. It was found that DISC1 has numerous binding partners, including NUDEL, FEZ1, activating transcription factor (ATF) 4/5, and microtubule-associated protein 1A (MAP1A).[15,17,18] NUDEL is a component of a pathway involved in cytoplasmic dynein movement, and is involved in neurofilament assembly, neuronal migration, and development of neurite morphology.[20-25] Overexpression of truncated DISC1 protein inhibits neurite outgrowth in PC12 cells, suggesting that the DISC1-NUDEL complex may be involved in neuronal outgrowth.[15,25,26] The hypothetical peptide product resulting from the Scottish translocation removes the interaction domain for NUDEL. The defective DISC1-NUDEL complex may be a cause of neurodevelopmental abnormalities in schizophrenia.[19] Recently, it has been shown that NUDEL oligopeptidase activity is under tight regulation through binding to DISC1, since a mutation very close to the DISC1-binding site of NUDEL abolishes this activity.[27] Interestingly, NUDEL cleaves a number of neuropeptides in vitro, some of which have previously been implicated in the pathophysiology of schizophrenia, including neurotensin (NT).[25,29] NT receptor agonists may be potential antipsychotics; thus, inhibition of NUDEL could lead to increase in local concentration of NT, which may have an antipsychotic effect.[27] Altered subcellular distribution of DISC1 has been reported in patients with psychosis and alcohol/substance abuse, with increased ratios of nuclear to cytoplasmic DISC1 protein levels in patients.[30] Cell culture studies in cortical neurons have found evidence that DISC1 may colocalize with mitochondrial markers, and that its subcellular targeting is independent of the NUDEL-binding site.[26] Hayashi et al[27] have also demonstrated that DISC1 and NUDEL bind in a neurodevelopmentally regulated manner and form a trimolecular complex with another protein, LIS1. LIS1 is involved in neuronal migration and corticogenesis. Although the function of this complex is currently unknown, it is thought to play a role in dynein-mediated motor transport.[27]

Another interacting partner of DISC1 is FEZ1, which is a mammalian homologue of the *Caenorhabditis elegans* UNC-76 protein, involved in axonal outgrowth and fasciculation. Miyoshi et al demonstrated that DISC1 participates in neurite extension through its C-terminal interaction with FEZ1.[31] The chromosomal location for *FEZ1* was previously implicated in a schizophrenia linkage analysis, although results from different populations

vary in significance.[32] A modest association between schizophrenia and *FEZ1* polymorphisms has been detected in a subset of Japanese patients.[33]

## Abnormalities in a *DISC1* pathway in schizophrenia

In our laboratory, we have tested the hypothesis that altered expression of *DISC1*, and/or its molecular partners *NUDEL*, *FEZ1*, and *LIS1* may underlie its pathogenic role in schizophrenia and explain its genetic association.[34] We examined the expression of *DISC1* and these selected binding partners in postmortem human brain. We found no difference in the expression of *DISC1* mRNA in schizophrenia, and no association with previously identified risk SNPs (all $F$ values <1.5, all $P$ values >0.2). *DISC1* immunoreactivity was significantly, albeit modestly (by approximately 20%), increased in the hippocampus of patients with schizophrenia: $F(1,73)=3.6$, $P=0.05$. However, the expression of *NUDEL*, *FEZ1*, and *LIS1* mRNA was each significantly reduced in schizophrenic tissue in both the dorsolateral prefrontal cortex and hippocampus and the expression of each gene showed association with a high risk *DISC1* polymorphism (all $P$ values <0.05).

These data implicate genetically linked abnormalities in the *DISC1* molecular pathway in the pathophysiology of schizophrenia. Given its role in brain development and plasticity via its interaction with a number of different proteins, *DISC1* remains a candidate gene for schizophrenia, and an understanding of its exact mechanistic role in neuronal pathways may shed more light on the disease.

## Conclusions

Schizophrenia is a devastating neuropsychiatric disorder, the genetics of which has been under extensive investigation for several decades. Despite being an exceedingly complex disease in terms of both etiology and pathogenesis, recent research is finally shedding light on schizophrenia susceptibility genes. There are several genes implicated by association studies and postmortem findings. Prominent among them are the genes *COMT*, *DTNBP1*, *GRM3*, *DISC1*, *NRG1*, *AKT1*, *GAD1*, *RGS4*, and *DRD2*. *DISC1* and its binding partners *FEZ1*, *NUDEL*, and *LIS1* are involved in cytoplasmic dynein movement, neurofilament assembly, neuronal migration, and neurite morphology, and may play a role in the neurodevelopmental deficits observed in schizophrenia.

Although the precise neurobiological cause of schizophrenia continues to be unknown, the abundance of evidence regarding susceptibility genes for schizophrenia cannot be dismissed. Identification of the molecular and cellular mechanisms that link susceptibility genes to the neurobiological functioning of the brain continues to be a major focus of research. As evidence for the functioning of the various susceptibility genes increases, it may be determined that these genes operate in a convergent molecular pathway affecting neural development and synaptic plasticity. The disruption of multiple genes within this pathway may lead to the development of schizophrenia. Such a convergent biochemical pathway may also be an attractive target for therapeutic intervention. ❑

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### *Genómica funcional en el cerebro humano postmortem: alteraciones de la vía molecular* DISC1 *en la esquizofrenia*

*Se ha identificado que el gen DISC1 (disrupted in schizophrenia 1) constituye una susceptibilidad genética para la esquizofrenia en base a datos provenientes de la clínica y de estudios de ligazón y de asociación de polimorfismo del nucleótido único (PNU), lo que sugiere que el riesgo de polimorfismos tendría su efecto en la estructura y función del hipocampo. Se ha propuesto la hipótesis que la alteración en la expresión del* DISC1 *y/o sus moléculas asociadas* (NUDEL [nuclear distribution element-like], FEZ1 [fasciculation and elongation protein zeta-1] y LIS1 [lissencephaly 1]) *podrían tener un papel patogénico en la esquizofrenia y explicar su asociación genética. Se ha examinado la expresión de* DISC1 *y sus elementos de unión en el hipocampo y la corteza prefrontal dorsolateral de cerebros humanos postmortem de pacientes esquizofrénicos y controles. No se encontraron diferencias en la expresión del RNAm del gen* DISC1 *en la esquizofrenia, ni tampoco asociación con los PNUs de riesgo identificados previamente. Sin embargo, la expresión de* NUDEL, FEZ1 *y* LIS1 *se encontró significativamente reducida en tejidos de sujetos esquizofrénicos, y la expresión de cada una de ellas mostró asociación con polimorfismos de* DISC1 *de alto riesgo. Estos datos sugieren que en la fisiopatología de la esquizofrenia existe un compromiso genético en la vía molecular del* DISC1.

### *Génomique fonctionnelle dans le cerveau humain postmortem : anomalies d'une voie moléculaire DISC1 dans la schizophrénie*

*La découverte du gène DISC1* (disrupted in schizophrenia gene) *consiste en la mise en évidence d'une susceptibilité génétique fondée sur une étude de liaison et sur une étude du polymorphisme d'un gène (single nucleotide polymorphism, SNP). Les études d'association suggèrent que les mutations cliniques ont un impact sur la fonction et la structure de l'hippocampe. Nous avons émis l'hypothèse qu'une expression altérée du gène DISC1 et/ou de ses molécules associées (NUDEL,* [nuclear distribution element-like]*, FEZ1,* [fasciculation and elongation protein zeta-1]*, et LIS1,* [lissencephaly 1]*) pourrait jouer un rôle dans la pathogènie de la schizophrénie, expliquant ses associations génétiques. Nous avons étudié l'expression du gène DISC1 et de ses éléments de liaison dans l'hippocampe et dans le cortex préfrontal dorsolatéral de cerveaux humains* postmortem *de patients schizophrènes et de témoins. Nous n'avons trouvé aucune différence dans l'expression de l'ARNm du gène DISC1 dans la schizophrénie, et aucune association avec des mutations uniques (SNP) identifiées antérieurement. Cependant, l'expression des gènes* NUDEL, FEZ1 *et* LIS1 *était réduite de façon significative dans le tissu provenant des patients schizophrènes, l'expression de chacun de ces gènes montrant une association avec la mutation unique (SNP). Ces résultats montrent que dans la physiopathologie de la schizophrénie il existe des anomalies génétiques de la voie moléculaire du gène* DISC1.


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