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ORIGINAL ARTICLE

Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia

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Disrupted in Schizophrenia 1 (DISC1) is a schizophrenia risk gene associated with cognitive deficits in both schizophrenics and the normal ageing population. In this study, we have generated a network of protein–protein interactions (PPIs) around DISC1. This has been achieved by utilising iterative yeast-two hybrid (Y2H) screens, combined with detailed pathway and functional analysis. This so-called 'DISC1 interactome' contains many novel PPIs and provides a molecular framework to explore the function of DISC1. The network implicates DISC1 in processes of cytoskeletal stability and organisation, intracellular transport and cell-cycle/division. In particular, DISC1 looks to have a PPI profile consistent with that of an essential synaptic protein, which fits well with the underlying molecular pathology observed at the synaptic level and the cognitive deficits seen behaviourally in schizophrenics. Utilising a similar approach with dysbindin (DTNBP1), a second schizophrenia risk gene, we show that dysbindin and DISC1 share common PPIs suggesting they may affect common biological processes and that the function of schizophrenia risk genes may converge.
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Introduction

Schizophrenia is a severe debilitating psychiatric disorder affecting ~1% of the population worldwide. The disease can be separated into three domains: (1) *positive symptoms*, characterised by hallucinations, delusions, thought disorder and paranoia; (2) *negative symptoms*, which include anhedonia, social withdrawal and thought poverty; (3) *cognitive symptoms*, with dysfunction in attention, working memory and executive function.[1] Current therapies are effective at treating positive symptoms but treatment of the negative and cognitive domains remain unmet medical needs.[1]

Although the aetiology of the disease remains unknown, several lines of evidence based on neuropathological, pharmacological and genetic data suggest that schizophrenia is a disease of the synapse.[2] Post mortem and brain imaging studies of schizophrenics reveal neuroanatomical pathologies such as smaller neuropil size, decreased number of dendritic spines and arborisations,[3,4] abnormal neuronal migration and orientation,[4–6] decreased number of synaptic proteins,[7,8] enlarged ventricles and decreased brain volumes.[2,9] These impairments suggest that abnormal or reduced synaptic connectivity may result from improper neurodevelopment culminating from an underlying genetic predisposition and/or early environmental insult.[2,10]

Further evidence that schizophrenia is a disease of the synapse comes from observations that *N*-methyl-D-aspartate receptor (NMDAR) antagonists, PCP and ketamine, induce a broad range of schizophrenic like symptoms in normal individuals and exacerbates symptoms in affected patients.[1] NMDA receptors also play a role in neuronal development and a transgenic mouse line, which has reduced expression of the NR1 subunit, has schizophrenic like behaviours.[11,12]

Recent advances in psychiatric genetics have identified an increasing number of schizophrenia risk genes including Disrupted in Schizophrenia 1

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(DISC1), neuregulin-1 (NRG1) and dysbindin (DTNBP1).[2] Disruption of the DISC1 gene by a balanced (1;11) (q42;q14) translocation was originally reported to co-segregate with major psychiatric illnesses in a Scottish family.[13] An increasing number of independent genetic studies have confirmed DISC1 as one of the most substantiated risk factors for schizophrenia.[2] Interestingly, all carriers of the translocation exhibit reduced P300 event related potential, a measurement of attention-dependent information processing that has been consistently shown to be impaired in schizophrenic patients.[14,15] Furthermore, a cys704ser polymorphism located on exon 10 of DISC1 is associated with reduced hippocampal grey matter and NAA signal, as well as abnormal engagement of the hippocampus during several cognitive tasks assayed by fMRI.[16] These observations suggest that DISC1 may participate in pathways important for cognitive function.[15]

The biological function of DISC1 remains poorly understood. However, recent reports that DISC1 interacts with Nudel (NDEL1) and PDE4B suggests roles for DISC1 in the regulation of neurodevelopmental and cAMP signalling pathways respectively.[17–20] These interactions are part of a larger effort to derive a network of DISC1 interactions, and were initially identified in the work described herein. Here, we report the 'DISC1 Interactome', which represents an interaction network consisting of 127 proteins and 158 interactions. Through detailed functional and pathway analysis, we provide evidence that DISC1 may be intimately linked to synapse function and that many of the proteins identified in our network are closely connected to genetic risk factors for schizophrenia such as dysbindin.[2] We performed a further set of Y2H screens with dysbindin, a presynaptic protein,[21] and have been able to show that it shares common protein–protein interactions (PPIs) with DISC1. This suggests even more strongly that there is the possibility of convergence in the biological function of schizophrenia risk genes, which may allow the identification of pathways that underlie the disease.

Materials and methods

Y2H library construction

Random cDNA libraries from human fetal brain poly(A^+) RNA (20 weeks, ResGen Carlsbad, CA, USA) and human Adult brain poly(A^+) RNA (Invitrogen: Discovery Line Human normal Brain mRNA, Sex: M, Age: 27, Catalog No: D6030-15, LOT No: A308079) were constructed in the pP6 plasmid derived from the original pACT2[22] and transformed in *E. coli* (DH10B; Invitrogen, Carlsbad, CA, USA). The complexity of the primary libraries was over 50 million clones. Sequence analysis was performed on 300 randomly chosen clones to establish the general characteristics of each library. The libraries were then transformed into yeast by classical lithium acetate protocol. Ten million independent yeast colonies were collected, pooled and stored at −80°C as equivalent aliquot fractions of the same library.[22]

Y2H bait plasmids

For C-terminal fusion, DISC1 was amplified using primers and cloned into a pLexA based plasmid (pBTM116 with minor modification) and a Gal4 based plasmid (pAS2ΔΔ with minor modification).[22] For N-terminal fusion, DISC1 was cloned into a pLexA based N-terminal plasmid and in a Gal4 based N-terminal plasmid (pB23). pB23 was obtained by insertion in *Sac*I−*Pst*1 of pAS2ΔΔ of two PCR products obtained using primers:

Gal4-5p: CGGGATTCGGT-GGTATGAAGCTACTGT CTTCTATCGAAC
Gal4-3p: TGCACTGC-AGTCACGGCGATACAGTCA ACTGTCTTTG
ADH-5p: TTTGCGGCCGCCATGCATTT-ACTTATA ATACAG
ADH-3p: CGCGAGCTCGAGATCCCGAGC from pA S2ΔΔ template.

All the constructs amplified by PCR have been fully sequenced using ABI 3700.

Two-hybrid screens were performed using a cell to cell mating protocol.[22] For each bait, a test screen was performed to adapt the screening condition. The selectivity of the HIS3 reporter gene was eventually modulated with 3-aminotriazole (Sigma, St Louis, MO, USA) in order to obtain a maximum of 285 histidine-positive clones for 50 millions diploids screened. For all the selected clones, lacZ activity was estimated by overlay assay on solid media in 96-well plate format. Inserts of all positive clones were amplified by PCR[22,23] and then sequenced on an ABI 3700 automatic sequencer (Applied Biosystem, Foster City, CA, USA).[22]

Screens for both DISC1 and the truncated DISC1 were performed against both human fetal and adult brain libraries. All other screens were performed against fetal brain library only (Table 1).

Prey identification

5′ and 3′ sequences were determined for all positive clones in a screen. These were in turn filtered for quality using PHRED and ALU repeats were masked. Sequence contigs were built using CAP3[24] and searched against the latest release of GenBank using BLASTN.[25]

Identifying reliable interactions

Interactions were filtered based on a predicted biological score (PBS).[22,23,26,27] The PBS was calculated based on randomly sequenced cDNA library and adopts the conventional form of a *P*-value, where the smaller the PBS (*P*-value) the more significant. Briefly, a background distribution of the library is estimated by random sampling. The cDNA library will contain different genes as well as several fragments for a given gene (redundancy) that are of different lengths and may span different portions of a

Table 1 Summary of yeast two-hybrid screens

Bait	*Vector*	*AA residues*	*Human brain library*	*Total number of interactions*	*High confidence interactions*
DISC1	pB27 (LexA, C-terminal fusion)	1–854	Fetal	134	16
DISC1	pB23 (no definition available) + pB29 (LexA, N-terminal fusion)	1–854	Fetal	140	26
DISC1 (N-term)	pB6 (GAL4, C-terminal fusion)	1–350	Fetal	379	39
DISC1	pB23 (GAL4, N-terminal fusion)	1–855	Adult	80	16
DISC1trunc	pB6 (GAL4, C-terminal fusion)	1–597	Adult	134	14
DISC1trunc	pB27 (LexA, C-terminal fusion) + pB6 (GAL4, C-terminal fusion)	1–597	Fetal	210	22
FLJ13386 (Cep63)	pB27 (LexA, C-terminal fusion)	1–542	Fetal	79	10
TRAF3IP1	pB28 (LexA, C-terminal fusion)	542–760	Fetal	123	17
NDEL1	pB27 (LexA, C-terminal fusion)	1–346	Fetal	81	17
SEC3L1	pB27 (LexA, C-terminal fusion)	1–895	Fetal	92	13
SH3BP5	pB27 (LexA, C-terminal fusion)	1–456	Fetal	58	2
TNIK	pB28 (LexA, C-terminal fusion)	321–507	Fetal	105	15
CDC5L	pB27 (LexA, C-terminal fusion)	1–803	Fetal	80	17
CDK5RAP3	pB31	1–507	Fetal	25	0
DTNBP1	pB27 (LexA, C-terminal fusion)	1–189	Fetal	97	17

Abbreviation: AA, amino acids.

given gene (diversity: each fragment is independent of the other). Both the redundancy and the diversity of the library are used in order to determine the likelihood that a given prey will interact with the bait of choice by chance assuming that each fragment has an equal probability to interact with the bait. An initial expectation value is calculated based on the size of the library (i.e. number of transcripts) and on the number of overlapping fragments that are found to interact with the bait. The greater the number of overlapping fragments the greater the likelihood that the observed interaction is real and not a chance event. Fragments that are out of frame or antisense are discarded.

The topology of the network can also affect the final PBS score. The interactions identified are also examined in context of the whole network. For example, if two proteins that interact also share other common interactions, the likelihood that interactions are real increases and the PBS is adjusted accordingly. Conversely, if a given prey is identified in several different and independent screens, above an established threshold, it is considered to be a false positive interaction. The final PBS score reflects the probability that the interaction occurs by chance and is divided into the following arbitrarily derived categories A < 1e-10 < B < 1e-5 < C < 1e-2.5 < D < 1 < E. The category E reflects sticky proteins that are found in several independent screens. The category D represents preys that only had one fragment identified. These may represent false positive interactions or may represent rare, low-copy number, transcripts in the library and require further validation. Interactions with PBS score in categories A–C are considered to be highly reliable.

Characterisation of over-represented biological themes
A 2×2 contingency table and Fisher's exact test were used to determine whether particular Gene Ontology (GO) terms,[28] InterPro Domains,[29,30] SWISSPROT Keywords,[31] and KEGG Pathways[32] were disproportionately represented in a given set of PPIs assuming a hypergeometric distribution.[29] The aim of this analysis is to determine if the observed frequencies (counts) in the set of interest (e.g. proteins that interact with DISC1) markedly differ from frequencies that would be expected by chance given a background distribution (i.e. human proteome or all genes expressed in the brain). Low *P*-values indicate that in the set of interest, a bias for a particular annotation (e.g. proteins belonging to microtubule based processes) is observed. GO terms for each gene were derived from Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), Proteome database (http://www.proteome.com/), and from InterPro domains. Annotation for each protein can be found in the Supplementary information.

A Monte Carlo simulation was also performed. We generated 1000 random networks each containing 127 brain expressed genes (the same number in the DISC1 network). For each of these random networks, over-represented biological process terms were identified as described above. A *P*-value for each of the observed terms in the DISC1 network was calculated as follows: Let n = biological process term with equal or better *P*-value in the random networks than observed (DISC1 network). Let m = total number of random networks.

$$\text{Simulation } P\text{-value} = \Sigma n/m$$

Brain expressed genes were derived from unpublished in house data.

PPI resources
Where possible, previously reported interactions were included in the over-representation analysis. These were derived from Entrez gene entries and from MINT, BIND and IntAct, PPI databases.[33–35] All interactions identified in this work have been deposited in the IntAct database (EBI-1104292 and EBI-1104295).[35]

PPI maps and matrix visualisation
PPI maps were generated using Cytoscape version 2.0.[36] Matrices were generated using Matrix2Png.[37]

Derivation of schizophrenia risk loci
Cytogenetic location for each gene was derived from Entrez Gene and compared to risk schizophrenia risk loci as reported in Online Mendelian Inheritance in Man (OMIM) (www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 181500).

Cytogenetic locations of chromosomal aberrations linked to schizophrenia were derived from a number of previously published articles.[20,38,39]

Pathway information and mapping
Pathway information was obtained using Pathway Miner[30] and Ingenuity, Inc. Pathway assist tool (www.ingenuity.com).

Results

DISC1 interacts with functionally diverse proteins
To assign a biological context for DISC1, a series of Y2H screens against human fetal and adult brain libraries were performed using the full-length DISC1 protein. Both GAL4 and LexA based Y2H screen methods were utilised, with different bait vectors containing either C-terminal or N-terminal fusion of the DNA binding domain (Table 1). The rationale behind screening both libraries with different vectors was to maximise the coverage of DISC1 interactors obtained and to increase our confidence in the reliability of the identified interactions.

A total of 289 interactors were identified in screens with the full length DISC1 protein. Of these, 34 were considered high-confidence interactions based on a method previously described[23] whereby a PBS score is assigned reflecting the likelihood of the interaction. Highly significant PBS scores have been shown to correlate with biologically relevant interactions confirmed in the literature and by other experimental systems.[20,26,27,40] Filtering on probability of interactions has been successful in removing false positives interactions and has been used in several different studies to generate reliable protein interaction maps and to deduce novel biological findings.[23,26,26,40,41]

In order to determine major biological themes related to the set of DISC1 interacting proteins, over-representation of GO terms was determined[28] (see Supplementary information). GO provides a set of terms used to describe gene products and is hierarchically divided into three main categories: biological processes, molecular function and cellular component. Biological processes are defined as one or more ordered assemblies of functions (e.g. cell growth and maintenance; pyrimidine metabolism). Cellular components refer generally to anatomical or macromolecular components in the cell (e.g. nucleus; ribosome complex). Molecular function corresponds to activity of gene products such as catalytic or binding activity (i.e. biochemical functions largely defined by the protein structure). The hierarchy of GO terms consists of more generic and less specific parent terms and more specific child terms. Using a cutoff of $P < 0.01$, proteins that interact with DISC1 can be broadly associated with cytoskeletal organisation and biogenesis, mRNA/protein synthesis, cell cycle/division, intracellular transport and signal transduction processes. These broad categories are used for clarity and the significant and specific GO terms within these categories can be seen in Figure 2as well as in the Supplementary Tables. Many of these proteins are localised to cellular components such as microtubule organising centre, microtubule and actin cytoskeleton, centrosome and cell cortex consistent with published reports of DISC1 subcellular localisation.[18,42,43] Proteins that interact with DISC1 have diverse functions and range from having enzymatic to structural roles. Over-represented molecular functions such as binding of cytoskeletal proteins, kinases and calmodulin suggest most of these proteins have a role in cell morphology through signal-transduction-mediated cytoskeletal organisation. Notably, many of these proteins such as Nudel, Lis1(PAFAH1B1), CDK5RAP3, the germinal center kinase TNIK and the Guanine exchange factor TRIO[44–48] have been implicated in neurodevelopmental related processes, consistent with other protein interaction studies that implicate DISC1 in neurodevelopment.[49] In addition, phosphodiesterase (PDE4B) and dystrophin (DMD) have been suggested to be important for cognitive function, deficits in which are a major component of schizophrenic symptomatology.[50,51]

Experimental derivation of the DISC1 network of interactions
In order to derive a PPI network around DISC1, a series of second round Y2H screens were performed. Eight DISC1 interacting proteins were selected for subsequent screening against fetal brain library (Figure 1a; Table 1). These proteins represented each of the main processes described above such as cell cycle (CDC5L, CDK5RAP3), signal transduction (SH3BP5, TNIK), transport/localisation (Nudel, SEC3L1, TRAF3IP1) as well as those associated with the centrosome (Nudel and FLJ13386). For each screen, only interactions meeting our cutoff criteria discussed above were used for further analysis (Supplementary information). None of the identified interactions from the CDK5RAP3 screen met the cut off criteria. An additional screen was performed with the N-terminal portion of DISC1 (AA 1–350) in order to see if there were region specific protein interactions, given that this region lacks coiled-coil domains[17] (Figure 1b).

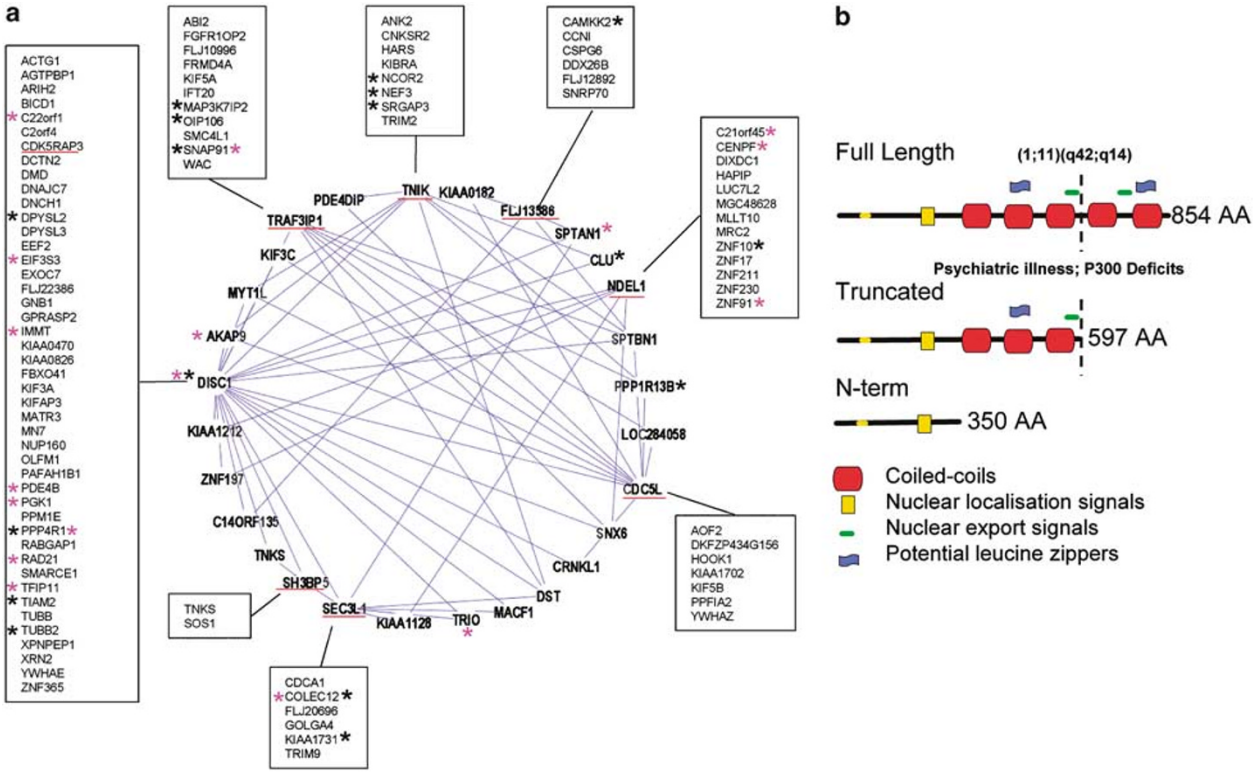


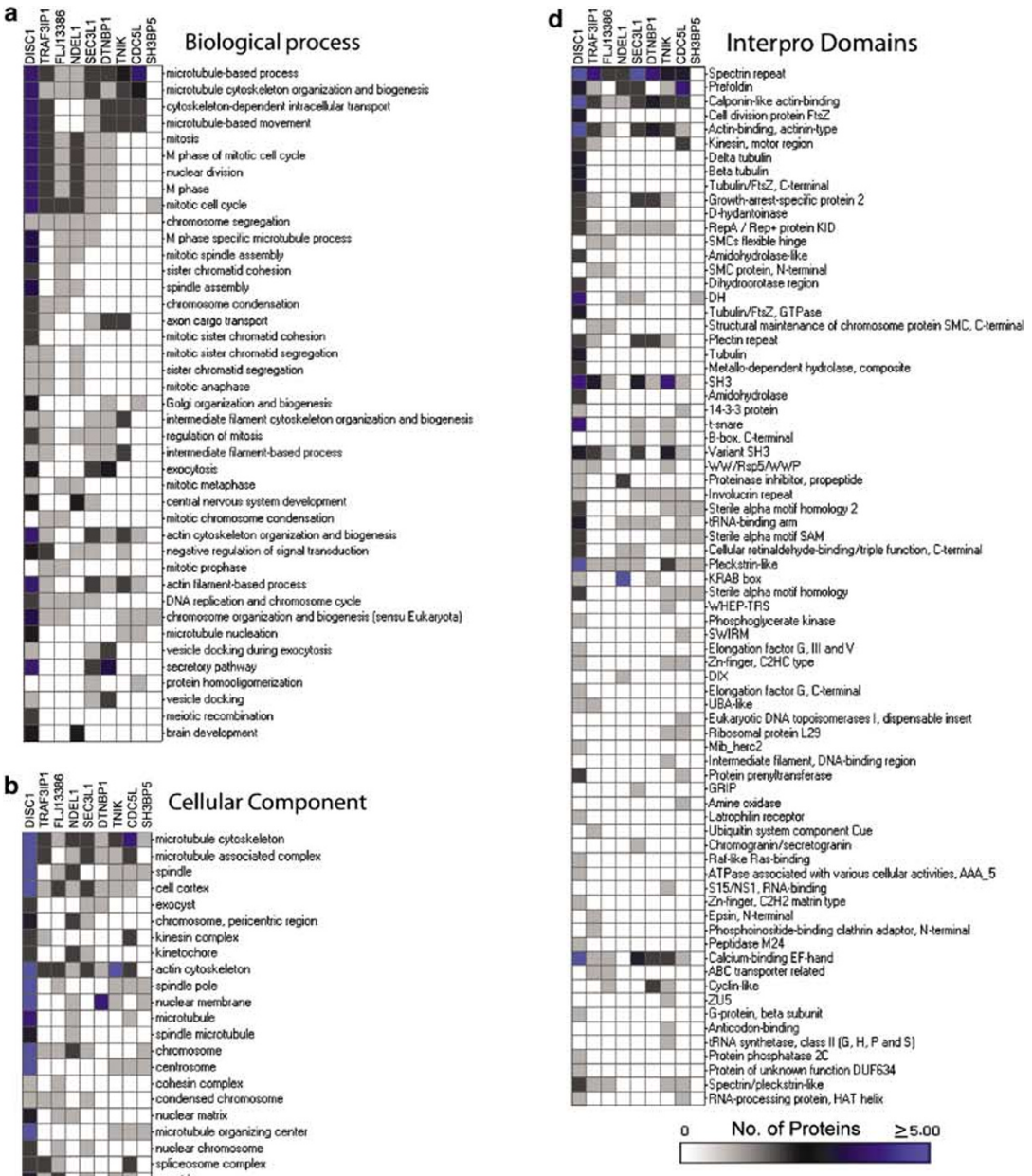
Figure 1 (**a**) DISC1 network of PPIs. Underlined in red are proteins that interact with DISC1 and were used as baits in further Y2H screens to derive the protein interaction network. Blue connecting lines indicates an interaction between molecules that interact with at least two proteins in the network. In boxes are proteins that interact *only* with the bait protein screened. *(black) indicates proteins that are located in a schizophrenia risk locus. *(magenta) indicates proteins that are found in regions where chromosomal abnormalities have been linked to schizophrenia.[36,37] (**b**) Schematic of DISC1 full-length protein, its potential truncated form and the N-terminus region.

In total, including previously described DISC1 screens, over 1097 interactions were identified. Conservative filtering for potential false positives, as described previously, resulted in a network of 127 proteins and 158 interactions, with the vast majority of interactions being novel. Results from each screen were then combined in order to build a physical connectivity graph (Figure 1a). For protein annotation and details on the likelihood of each interaction, as described by the PBS, see Supplementary information. Common interactions between DISC1 and molecules used in the second round screens were identified (Figure 1a). For example, the synaptic protein spectrin (SPTBN1) was identified as an interacting protein to DISC1, CDC5L, FLJ13386(Cep63), TRAF3IP1 (MIP-T3), SH3BP5 and TNIK. KIAA1212, a protein predicted to be downstream of Wnt signalling, was identified in both Nudel and DISC1 screens. The presence of common interaction clusters suggests that these proteins are either in the same complex or are part of the same functional process in the cell.[40,41,52,53]

Biological context of the DISC1 PPI network

As for DISC1, over-representation analysis of GO terms was determined for the derived network and for each individual screen (see Materials and methods). Most of the interactions identified for Nudel, TNIK, TRAF3IP1, CDC5L, SEC3L, FLJ13386 and SH3BP5 are novel. Nonetheless, the over-represented GO terms are in line with known biological processes and subcellular compartments to which these proteins

Figure 2 (**a**) Biological processes, (**b**) molecular functions, (**c**) cellular components and (**d**) protein families over-represented in the DISC1 network. Terms (rows) are ranked based on over-representation in the whole network using a cutoff of $P<0.01$ (Supplementary information). Shading highlights the number of proteins from each respective screen (columns) that belong to a given GO annotation or contain a particular InterPro domain (rows). White (no proteins) to blue ($\geqslant 5$ proteins) in a given category per screen. Common themes were identified amongst each screen. Proteins that interact with DTNBP1 participate in the same processes as proteins in the DISC1 PPI network suggesting a convergent theme among these schizophrenia risk factors.


a
Biological process
DISC1
TRAF3IP1
FLJ13386
NDEL1
SEC3L1
DTNBP1
TNIK
CDC5L
SH3BP5
microtubule-based process
microtubule cytoskeleton organization and biogenesis
cytoskeleton-dependent intracellular transport
microtubule-based movement
mitosis
M phase of mitotic cell cycle
nuclear division
M phase
mitotic cell cycle
chromosome segregation
M phase specific microtubule process
mitotic spindle assembly
sister chromatid cohesion
spindle assembly
chromosome condensation
axon cargo transport
mitotic sister chromatid cohesion
mitotic sister chromatid segregation
sister chromatid segregation
mitotic anaphase
Golgi organization and biogenesis
intermediate filament cytoskeleton organization and biogenesis
regulation of mitosis
intermediate filament-based process
exocytosis
mitotic metaphase
central nervous system development
mitotic chromosome condensation
actin cytoskeleton organization and biogenesis
negative regulation of signal transduction
mitotic prophase
actin filament-based process
DNA replication and chromosome cycle
chromosome organization and biogenesis (sensu Eukaryota)
microtubule nucleation
vesicle docking during exocytosis
secretory pathway
protein homooligomerization
vesicle docking
meiotic recombination
brain development
b
Cellular Component
DISC1
TRAF3IP1
FLJ13386
NDEL1
SEC3L1
DTNBP1
TNIK
CDC5L
SH3BP5
microtubule cytoskeleton
microtubule associated complex
spindle
cell cortex
exocyst
chromosome, pericentric region
kinesin complex
kinetochore
actin cytoskeleton
spindle pole
nuclear membrane
microtubule
spindle microtubule
chromosome
centrosome
cohesin complex
condensed chromosome
nuclear matrix
microtubule organizing center
nuclear chromosome
spliceosome complex
spectrin
cortical actin cytoskeleton
cortical cytoskeleton
extrinsic to plasma membrane
insoluble fraction
extrinsic to membrane
cell-cell adherens junction
apicolateral plasma membrane
cell junction
intercellular junction
c
Molecular Function
DISC1
TRAF3IP1
FLJ13386
NDEL1
SEC3L1
DTNBP1
TNIK
CDC5L
SH3BP5
structural constituent of cytoskeleton
motor activity
tubulin binding
dynein binding
microtubule motor activity
intracellular transporter activity
actin binding
small GTPase regulatory/interacting protein activity
hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amides
GTPase regulator activity
clathrin binding
kinase binding
cytoskeletal adaptor activity
guanyl-nucleotide exchange factor activity
calmodulin binding
d
Interpro Domains
DISC1
TRAF3IP1
FLJ13386
NDEL1
SEC3L1
DTNBP1
TNIK
CDC5L
SH3BP5
Spectrin repeat
Prefoldin
Calponin-like actin-binding
Cell division protein FtsZ
Actin-binding, actinin-type
Kinesin, motor region
Delta tubulin
Beta tubulin
Tubulin/FtsZ, C-terminal
Growth-arrest-specific protein 2
D-hydantoinase
RepA / Rep+ protein KID
SMCs flexible hinge
Amidohydrolase-like
SMC protein, N-terminal
Dihydroorotase region
DH
Tubulin/FtsZ, GTPase
Structural maintenance of chromosome protein SMC, C-terminal
Plectin repeat
Tubulin
Metallo-dependent hydrolase, composite
SH3
Amidohydrolase
14-3-3 protein
t-snare
B-box, C-terminal
Variant SH3
WW/Rsp5/WWP
Proteinase inhibitor, propeptide
Involucrin repeat
Sterile alpha motif homology 2
tRNA-binding arm
Sterile alpha motif SAM
Cellular retinaldehyde-binding/triple function, C-terminal
Pleckstrin-like
KRAB box
Sterile alpha motif homology
WHEP-TRS
Phosphoglycerate kinase
SWIRM
Elongation factor G, III and V
Zn-finger, C2HC type
DIX
Elongation factor G, C-terminal
UBA-like
Eukaryotic DNA topoisomerases I, dispensable insert
Ribosomal protein L29
Mib_herc2
Intermediate filament, DNA-binding region
Protein prenyltransferase
GRIP
Amine oxidase
Latrophilin receptor
Ubiquitin system component Cue
Chromogranin/secretogranin
Raf-like Ras-binding
ATPase associated with various cellular activities, AAA_5
S15/NS1, RNA-binding
Zn-finger, C2H2 matrin type
Epsin, N-terminal
Phosphoinositide-binding clathrin adaptor, N-terminal
Peptidase M24
Calcium-binding EF-hand
ABC transporter related
Cyclin-like
ZU5
G-protein, beta subunit
Anticodon-binding
tRNA synthetase, class II (G, H, P and S)
Protein phosphatase 2C
Protein of unknown function DUF634
Spectrin/pleckstrin-like
RNA-processing protein, HAT helix
0
No. of Proteins
≥5.00

have been associated and are consistent, as expected, with over-represented themes from the DISC1 set of interactions. Figure 2a–d illustrates the most significant biological processes, cellular components, molecular functions and protein domains for the network ranked by significance $P<0.01$ (over-represented themes for each individual screen can be found in Supplementary information). Proteins in the network share many common biological processes and subcellular localisation, suggesting greater confidence in the reliability of these interactions.[40,41,52] Over-represented biological processes include regulation/organisation of actin and microtubule cytoskeleton, cell cycle/division and intracellular transport. Many of these processes are involved in dendritic/axonal morphology, synaptic connectivity, and communication. In terms of cellular components, most proteins are localised to the nucleus, actin/microtubule cytoskeleton, cell cortex, kinetochore, chromosome and cytoplasm. Molecular functions over-represented in the network suggest a bias in the network for proteins that are mainly involved in cytoskeletal regulation and stability.

The fact that proteins in our network share common interactions as well as biological processes, strongly suggests that these interactions are real as opposed to random. Nonetheless, in order to test whether a random sample of brain expressed genes would contain the same biases as our network, we generated 1000 random networks each consisting of 127 proteins. Brain expressed genes were derived from unpublished in-house microarray data. The null assumption is that randomly selected genes that are expressed in the brain can be arranged in a similar network and with the same biases in biological processes as observed in the DISC1 network (e.g. cytoskeleton). For each respective random network, we determined the over-represented themes as described and calculated the number of times a biological process term identified in the random set was equally or more significant than the values identified in the DISC1 network (see Materials and methods). Table 2 shows the *P*-value for each biological process term in our network suggesting that our observations are not random and due to an intrinsic brain bias. These observations also highlight that the clustering of functional groups in our network is not random and enforces the PBS for the protein interactions. Furthermore, this observation confirms previous findings that, as expected, proteins form functional modules in interaction networks.[53–56]

The DISC1 interactome suggests a role for DISC1 in synapse development

If DISC1 is to have an effect on cognitive processes and synaptic pathology, then it must do so by affecting genes or pathways that are implicated in neurodevelopment and plasticity such as BDNF, Wnt and Reelin, and the regulation of members of the RhoGTPase family.[2,41] In order to test this hypothesis, we searched the literature and protein–protein interaction/pathways databases in order to see if proteins in our network were involved in critical processes of synapse development, formation and maturation. In addition to the well-documented interactions with Lis1, Nudel and FEZ1, which implicate DISC1 in neuronal migration, many of the proteins in our network are involved in pathways critical for the formation of synapses namely synaptic activity mediated by the glutamatergic AMPAR and NMDAR receptor complexes, dendritic–axonal contact (e.g. Integrins, receptor tyrosine kinases, Cadherins), glial secreted factors (e.g. TNF-α and Wnt), regulation of actin dynamics (e.g. regulation of RhoGTPases by SRGAP3,KARLN, TRIO and SOS1), cytoskeletal stability (e.g. microtubule and dendritic stability: spectrins, ankyrin, microtubule crosslinking factors and neurofilament proteins), intracellular transport (e.g. dendritic targeting of mRNA, proteins and glutamate receptors), neuronal polarity and migration (e.g. reelin, Robo-slit signalling pathways), dendritic growth and branching by neurotrophic factors via Trk and EGF receptors (e.g. SOS1 and SNX6), gene expression (e.g. NCOR2 and AOF2), proteins synthesis (e.g. EIF3S3 and EEF2) and neuronal apoptosis (e.g. JNK signalling cascade, CLU, SH3BP5, PPP1R13B, RAD21) (Figure 3a–d).[41–49] These are all processes that are critical for the development, maturation and plasticity of synapses, and which may also be linked to schizophrenia-related neuropathologies.[57–65] Furthermore, a recent publication has systematically identified genes required for synapse structure in function in *Caenorhabditis elegans* neuromuscular junction.[66] Proteins in our network identified in this study include SOS1, DMD, SPTAN1 and YWHAZ.[66]

Potential implications of the DISC1(1;11) translocation

The translocation leading to the disruption of the DISC1 gene in the Scottish family may result in haploinsufficiency or in the production of a truncated protein product.[67] A truncated protein may gain and/or lose certain protein interactions, in doing so it may act in a dominant-negative manner. In order to explore these potential outcomes, we screened both adult and fetal brain libraries using the putative truncated form (AA1–527) of the protein (Figure 4 and 1b). As described previously, we selected 31 high confidence interactions of truncated DISC1 and identified the proteins that may potentially contribute to each of the scenarios as described above (Figure 4). Using proteins from each set, we mapped them to their equivalent GO categories to see what processes, molecular functions and cellular components are affected due to translocation (see Supplementary information). Interestingly, some of the processes affected by the loss of function are also affected by the gain of function such as proteins involved in microtubule-based processes, cell division, actin dynamics, Rho protein signal transduction and cytoskeleton-dependent intracellular transport (see Supplementary information).

Table 2 *P*-values reflect likelihood of each term being significant in a random network

Biological process term	*Monte Carlo* P-*value*
Microtubule-based process	<0.001
Microtubule cytoskeleton organisation and biogenesis	<0.001
Cytoskeleton-dependent intracellular transport	<0.001
Microtubule-based movement	<0.001
Mitosis	<0.001
M phase of mitotic cell cycle	<0.001
Nuclear division	<0.001
M phase	<0.001
Mitotic cell cycle	<0.001
Chromosome segregation	<0.001
M phase specific microtubule process	<0.001
Mitotic spindle assembly	<0.001
Sister chromatid cohesion	<0.001
Spindle assembly	<0.001
Chromosome condensation	<0.001
Axon cargo transport	0.001
Mitotic sister chromatid cohesion	<0.001
Mitotic sister chromatid segregation	0.002
Sister chromatid segregation	0.002
Mitotic anaphase	0.001
Golgi organisation and biogenesis	<0.001
Intermediate filament cytoskeleton organisation and biogenesis	0.003
Regulation of mitosis	0.004
Intermediate filament-based process	0.003
Exocytosis	0.009
Mitotic metaphase	0.003
Central nervous system development	<0.001
Mitotic chromosome condensation	0.001
Actin cytoskeleton organisation and biogenesis	<0.001
Negative regulation of signal transduction	0.012
Mitotic prophase	<0.001
Actin filament-based process	<0.001
Dna replication and chromosome cycle	0.023
Chromosome organisation and biogenesis (sensu Eukaryota)	<0.001
Microtubule nucleation	0.013
Vesicle docking during exocytosis	0.01
Secretory pathway	<0.001
Protein homooligomerisation	0.017
Vesicle docking	0.01
Meiotic recombination	0.018
Brain development	0.014

These results support the observed biological processes bias in the DISC1 network.

We also performed screens with the N-terminal portion of DISC1 (Figure 1b), where we expected to identify similar interaction partners to the truncated protein. Interestingly, there was little overlap between these screens, only six interactions in common, indicating that the additional 177 AA found in the truncated protein, which contains coiled-coil regions, may interfere with proteins that bind to the N-terminal region of DISC1.

Most of the processes previously described relating to synapse formation could be affected by loss and gain of interactions. However, the loss and gain of interactions as a result of truncation described here could be related to screen efficiency as well as to the choice of vectors used. Therefore, these differences need to be further validated in other experimental systems. Nonetheless, these observations highlight potential regions of interaction within the DISC1 protein and suggest that the C-terminal portions of the protein may constrain or interfere with proteins that interact with the N-terminal region.

Schizophrenia risk genes share common protein interactions

Several proteins in our network are in schizophrenia-associated loci, as defined by OMIM, or are in regions of documented chromosomal aberrations linked to schizophrenia (Figure 1a) suggesting that DISC1 and other risk genes may converge on similar biological processes. Given this observation, it was important to see if we could link proteins identified in the DISC1 network to other genes implicated in schizophrenia. To this end, additional Y2H screens with another major schizophrenia risk gene DTNBP1 (Table 1)[2] was conducted. These screens identified 17 high confidence interactors (Table 1; Figure 5). Intriguingly, like DISC1, dysbindin interacts with microtubule crosslinking factors MACF1 and DST (Figure 5). Dysbindin also shares common interactions with other proteins in our network such as SEC3L1 (exocyst complex), CDC5L (cell cycle regulation), TNIK (germinal center kinase) and TRAF3IP1 (protein associated with cytokine signalling) (Figure 1a). As described, GO over-representation analysis was performed in the dysbindin set of protein interactions. Similar themes as identified in the DISC1 interactome were found in the set of dysbindin interactions such as cell division, microtubule organisation and biogenesis, axon cargo transport and exocytosis (Figure 3a–d).

Discussion

In this study, we have generated a network of PPIs around a schizophrenia risk factor, DISC1, in order to identify potential pathways affected by this protein that in turn may be central for understanding the molecular pathology of schizophrenia. Not only have we identified a large number of novel interacting partners for DISC1, we have also found a plethora of previously unknown interactions for seven of its direct interactors. Through this iterative set of yeast-two hybrid screens, in combination with literature and pathway database mining, we have further implicated DISC1 in the neurodevelopmental hypothesis of schizophrenia[2,10,67] while for the first time providing evidence from our network that DISC1 is likely to participate in synapse formation and maturation,[57–65] potentially as a key 'hub' protein in these processes.[40]

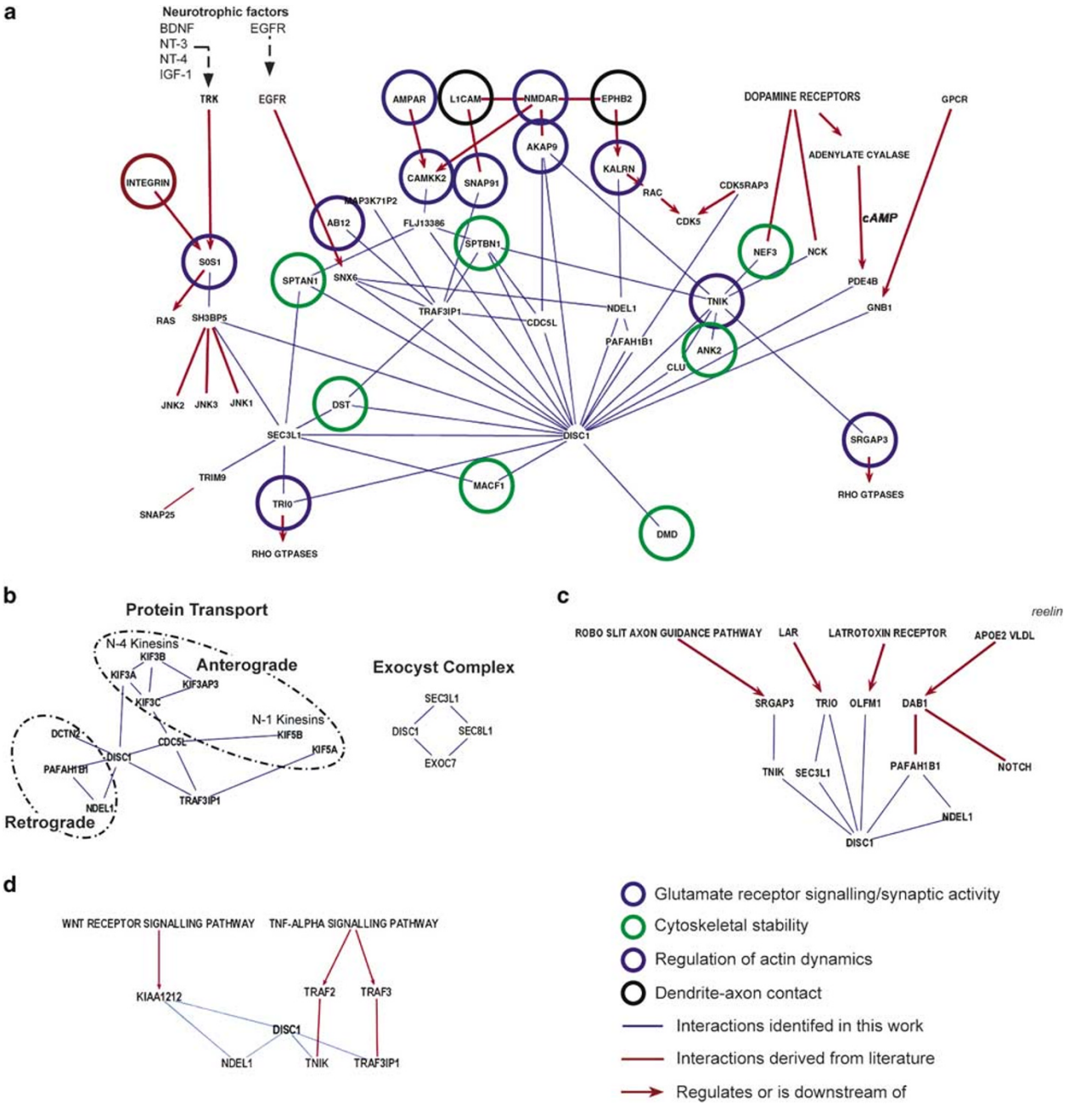


Figure 3 Potential relationship between the DISC1 network and synapse development. Schematic diagram showing sections of the DISC1 interactome that show evidence of a connection between DISC1 and key processes involved in synapse development/maturation, which in turn may underline observed neuroanatomical pathologies in schizophrenia. (**a**) DISC1 interacts with proteins involved in key processes involved in the proper formation of synapses. Deficits in these processes may underlie decreased dendritic branching, arborisations and neuropil size observed pathologically.[3,4] (**b**) DISC1 interacts with proteins involved in both retrograde (dyenin associated) and anterograde (kinesin associated) transport as well as components of the exocyst complex, which are important for the trafficking of glutamate receptors. Deficits in these processes may result in a decrease in the number of synaptic proteins seen in schizophrenics.[8,74] (**c**) DISC1 interacts with proteins downstream of neuronal migration signalling pathways. Deficits in these pathways could result in the disarray of neuronal orientation and abnormal axon projections seen in schizophrenics.[5,6] (**d**) Glial secreted factors such as Wnt and TNF-α also play an important role in synaptogenesis.[58]

To date, several lines of evidence implicate DISC1 not only in schizophrenia but also in cognition.[16,68,69] *In vitro, in vivo* and clinical data suggest that deficits in DISC1 could result in abnormal neuronal migration, faulty dendritic development and synapse maturation, and thus ultimately cognitive deficits. DISC1 has been localised to dendritic spines and overexpression of the putative truncated form re-

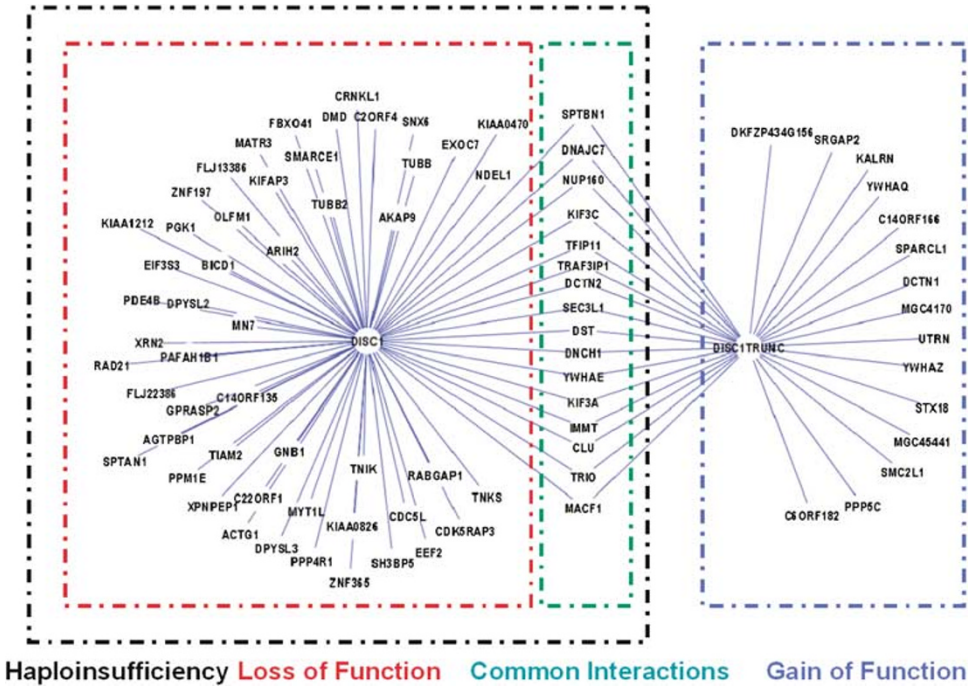


Figure 4 Representation of the potential effects of the (1;11) translocation on the DISC1 interactome. The translocation may result in haploinsufficiency or in the production of a truncated protein product.[67] A truncated protein may gain and/or lose certain protein interactions, in doing so it may act in a dominant-negative manner. Haploinsufficiency: DISC1 protein is not expressed. Loss/Gain of function: the loss of the C-terminus results may result in the ability of DISC1 to interact with new partners as well as lose C-terminal dependent protein interactions. Furthermore, the dominant-negative form may compete for binding with wild type form. It is not known whether the truncated protein is expressed in patients with the translocation.

sulted in abnormal neurite outgrowth.[19] Work by Kamiya *et al*.[70] shows that *in utero* RNAi depletion of DISC1 (analogous to haploinsufficiency model) results in abnormal neuronal migration, thinning of dendritic spines, as well as decreased number of dendritic arborisations. The effect of the (1;11) translocation at the behavioural level has been more compelling with all carriers presenting measurable cognitive deficits.[15] Furthermore, polymorphisms in the DISC1 gene have been associated with poorer performance in neurocognitive tasks in schizophrenics, and lower cognitive ability in the normal aging population.[16,68,69] Although it is not yet known whether any (1;11) translocation carriers exhibit synaptic pathologies, such as reduced number of dendrites or abnormal connectivity, the DISC1 interactome, derived in this study, suggests that abnormalities in the DISC1 protein could have repercussions for biological processes critical for synapse development/maturation, biochemical signalling and hence cognitive performance (Figure 3a–d).

The consequence of the (1;11) translocation at the protein level for DISC1 is still unknown and the presence of a truncated protein has not been confirmed in the Scottish (1;11) family. Nonetheless, studies using the truncated DISC1 protein may provide an informative model to understand the underlying pathology. Using our network, we would predict perturbation of a common set of biological processes whether a truncated protein is produced or a haploinsufficiency model occurs. This would principally involve proteins involved in cytoskeletal stability and organisation, intracellular transport and cell-cycle/division. Intriguingly, this is supported by *in vivo* overexpression of truncated DISC1 (analogous to production of truncated DISC1) results in a very similar phenotype of defective neuronal migration.[70]

Is there a convergence in processes affected by schizophrenia risk factors? Recent evidence has demonstrated that proteins within the same disease are more likely to interact or belong to the same functional modules in biological networks.[71] Many proteins in the DISC1 network are in a chromosomal region associated with schizophrenia or associated, through pathway analysis, with proteins implicated in schizophrenia indicating that DISC1 may interact with and/or participate in the same biological processes as other schizophrenia risk factors (Figures 1, 2 and 5). Interestingly, dysbindin, another major schizophrenia risk factor (2; 21) shares common protein interactions with DISC1. Both DISC1 and dysbindin interact with the microtubule crosslinking factors dystonin (DST/BPAG1) and microtubule-actin crosslining factor 1 (MACF1) (Figure 5). These microtubule crosslinking factors, also known as plankins, interconnect all three cytoskeletal filament networks: actin filaments, microtubules and inter-

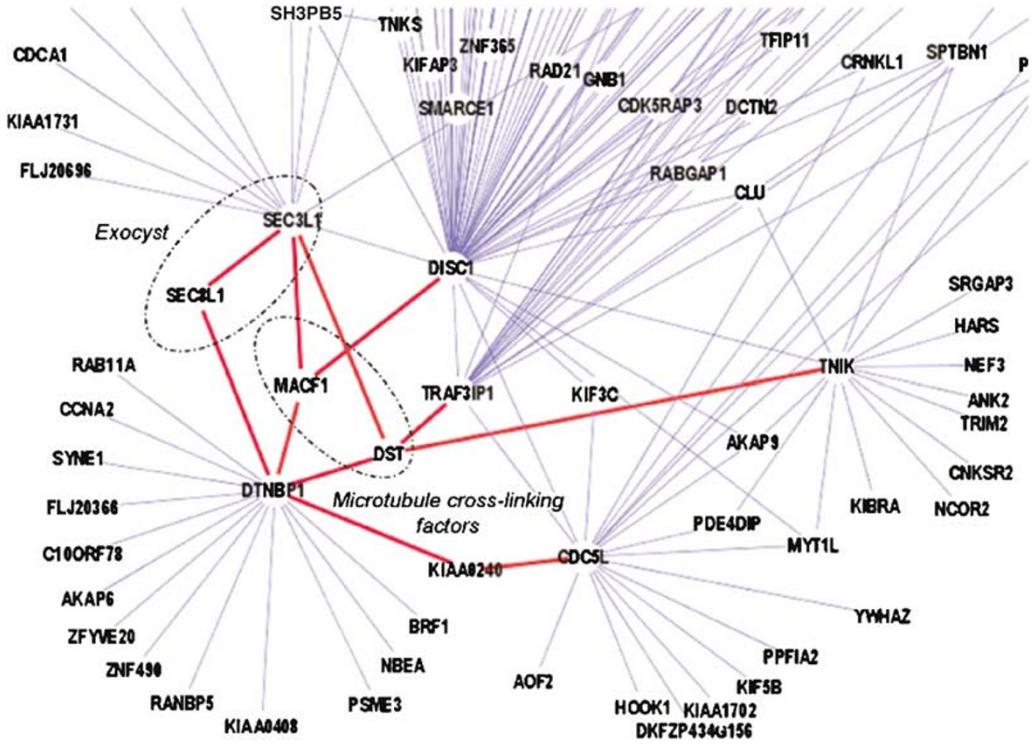


Figure 5 Two major schizophrenia risk factors, DISC1 and DTNBP1, share common interactions. Dysbindin shares common PPIs with DISC1 and other proteins in the DISC1 network (red edges). DST and MACF1 are microtubule crosslinking factors that interconnect all three cytoskeletal filament networks: actin and intermediate filaments as well as microtubules. Both DISC1 and dysbindin also interact with members of the exocyst complex, which are involved in the transport of glutamate receptors. In addition to sharing common protein interactions with DISC1, dysbindin interacts with proteins in similar biological processes that were enriched in the DISC1 network (see Figure 2).

mediate filaments. Reduction in dendritic branching is observed in MACF1 (*kakapo*) mutants in *Drosophila* and variants of DST have been shown to be essential for neuronal survival. Furthermore, DST deficient neurons are defective in axonal transport due to unstable and disorganised microtubules.[72,73] Both DISC1 and dysbindin also interact with members of the exocyst complex, which play a role in trafficking of glutamate receptors to synaptic terminals and it would be interesting to see how deficits on either of these proteins will affect glutamatergic transmission and receptor density (Figures 1a and 3b). These observations suggest that both DISC1 and dysbindin may affect structure and function of synapses by disruption of intracellular transport and cytoskeletal stability. These findings also highlight that the increasing set of schizophrenia risk genes may actually converge functionally on a more rational set of processes, which may contribute to the observed deficits in connectivity observed in schizophrenic patients.[2,71] This would be of great value to the psychiatric research community as it starts to make sense of this ever increasing number of candidates.

There are many challenges involved in the analysis of protein interaction networks. First, is the careful consideration and reliability in the observed interactions. In order to account for potential false positives, we adhered to a stringent set of criteria that relied on the likelihood of interactions being observed by chance given the distribution of the cDNA library. Furthermore, many of the proteins in our network share common interactions suggesting the presence of interaction clusters, which would not occur in a random network.[56] The consistency in protein annotation across all screens indicate that these proteins belong to common biological processes and therefore suggests that the network is organised and non-random consistent with previous findings.[53–55] In order to test this, we showed that (1) proteins in our network are biased to the same biological processes when comparing to a background distribution of brain expressed genes and (2) randomly generated networks of brain expressed genes are not biased to the same biological processes observed in the DISC1 network. However, many proteins in the network are of unknown function and therefore other biological properties may have been overlooked. Furthermore, many of these interactions may be brain region specific, cellular compartment dependent and/or favoured under a particular stimulus. In addition, interactions also need to be characterised in spatial and temporal contexts. There is already a precedent with DISC1 for interactions to be under developmental control as shown by the transient interaction with Nudel.[17]

In generating this network of PPIs around DISC1, we provide a rich data set that can be used by a wider audience to guide their research into not only DISC1 but schizophrenia more generally. For example, previously reported DISC1 interactions such as those to Nudel, Lis1 and PDE4B were originally derived from the screens described in this work and have been subsequently substantiated *in vivo*.[17,20] It will be critical to expand this experimental set to many of the novel interactions identified in this present study. We hope that we have provided for the first time a molecular framework by which the mechanisms of schizophrenia can be unravelled, using DISC1 as our key protein landmark. Moreover, the data set may allow the field to move away from receptor-based therapy to potentially more focused intracellular therapeutic targets.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)