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DISC1: Structure, Function, and Therapeutic Potential for Major Mental Illness

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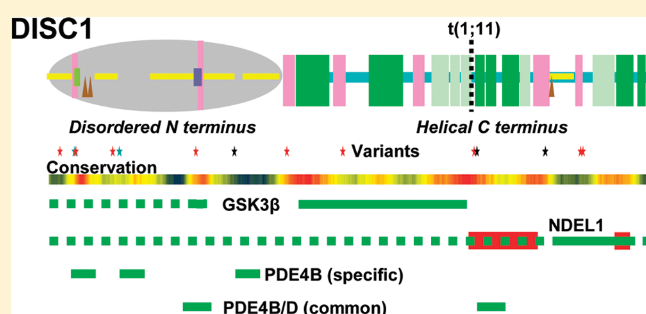
Supporting Information



ABSTRACT: *Disrupted in schizophrenia 1 (DISC1)* is well established as a genetic risk factor across a spectrum of psychiatric disorders, a role supported by a growing body of biological studies, making the DISC1 protein interaction network an attractive therapeutic target. By contrast, there is a relative deficit of structural information to relate to the myriad biological functions of DISC1. Here, we critically appraise the available bioinformatics and biochemical analyses on DISC1 and key interacting proteins, and integrate this with the genetic and biological data. We review, analyze, and make predictions regarding the secondary structure and propensity for disordered regions within DISC1, its protein-interaction domains, subcellular localization motifs, and the structural and functional implications of common and ultrarare *DISC1* variants associated with major mental illness. We discuss signaling pathways of high pharmacological potential wherein DISC1 participates, including those involving phosphodiesterase 4 (PDE4) and glycogen synthase kinase 3 (GSK3). These predictions and priority areas can inform future research in the translational and potentially guide the therapeutic processes.

KEYWORDS: DISC1, schizophrenia, structure, bioinformatics, GSK3β, therapeutic potential

Disrupted in schizophrenia 1 (DISC1), a protein originally identified from a gene directly disrupted by a balanced translocation in a large Scottish family with a high loading of major mental illness,[1,2] is now established by considerable genetic evidence as a risk factor for a wide array of psychiatric illnesses including schizophrenia, bipolar disorder, major depression, and potentially autism spectrum disorders (reviewed in refs 3 and 4). Recently, the term "DISCopathies" was coined to classify brain disorders related to dysfunctional DISC1 protein as a single disease entity.[5] DISC1 is present at the intersection of several neurodevelopmental pathways, one or more of which affect major mental illness pathology. It acts as a scaffold, binding a number of other proteins several of which have been shown to be independent risk factors for major mental illness (reviewed in ref 4).

The DISC1 protein interaction network therefore makes an attractive target for future therapeutic intervention,[6] and already some evidence shows DISC1 to be affected by known therapeutic drugs, with the atypical antipsychotics olanzapine and risperidone altering *DISC1* transcript levels in the mouse brain.[7] Additionally, depression and schizophrenia-related phenotypes seen in two mouse models of DISC1 mutation are partially restored by the antidepressant bupropion or the antipsychotics haloperidol and clozapine, respectively.[8] Furthermore, these mental-illness-related phenotypes are also treated in part by inhibitors of glycogen synthase kinase 3β (GSK3β),[9,10] a DISC1 interacting protein whose other inhibitors include the potent mood stabilizer, lithium.[11] While it therefore appears that DISC1 acts on known therapeutic pathways, it is perhaps even more exciting that variation in DISC1 may be associated with forms of schizophrenia resilient to treatment.[12] While these results have yet to be successfully replicated,[13] they imply that DISC1 may provide a target for the development of treatments for some of the sufferers of major mental illness for whom current pharmaceuticals are of limited or no use.

The DISC1 protein itself, however, at present, represents a difficult drug target due to lack of a known or anticipated enzymatic activity and the absence of a solved crystal structure. A recent report demonstrated that DISC1 function can be dynamically regulated via phosphorylation,[14] highlighting the possibility for the development of pharmacotherapies directly targeting DISC1. Any DISC1-based therapeutic molecule would most likely have to either modulate interaction of DISC1 with one of its many protein binding partners, the design of which would be greatly facilitated by a detailed understanding of the

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three-dimensional (3-D) structure of DISC1 to aid rational drug discovery, or instead affect those DISC1-binding proteins directly. In this review, we therefore present a critical summary analysis of the current understanding of the DISC1 protein structure by previously undertaken biophysical studies and by bioinformatics. We provide a comprehensive overview of DISC1 binding regions, subcellular localization, and known sequence variants implicated in psychiatric illness. Finally, we review in detail the DISC1-related protein signaling pathways, which currently show the most potential for future therapeutic intervention.

1. STRUCTURAL INFORMATION ON DISC1

Even though *DISC1* was first linked to major mental illness in 2000,[2] and its importance subsequently confirmed and replicated in numerous independent genetic studies,[3,4] no full-length or even partial/fragment experimental 3-D structures have been forthcoming. Indeed, to date, biophysical characterization of the full-length protein is still almost totally lacking.[3] In the absence of structural information for DISC1, experimental work has relied on shorter constructs and domain delineation based on sequence analysis.[15−17] This lack of structural information leaves a serious information gap for researchers in understanding the underlying effects of missense mutations and single nucleotide polymorphisms (SNPs) that have been linked to susceptibility to disease. Are experimental results ascribed to partial constructs physiologically relevant or informative with respect to a fully folded, functional DISC1? How does sequence variation in *DISC1* impact on the structure and function of the expressed protein? How do the various SNPs and mutations contribute toward the risk of developing mental illness? Will the structure suggest routes to the development of therapeutic intervention?

The full-length DISC1 sequence has been suggested to consist of two regions: (i) an N-terminal "head" domain spanning amino acid residues ∼1−350, that does not share homology to any known fold/s, and (ii) a C-terminal coiled-coil region (spanning ∼350−854) that shows greater conservation among orthologs than the N-terminus.[2,3,17,18] Via a comprehensive bioinformatics approach, we review and refine the secondary structure of DISC1.

1.1. The Case for Protein Disorder in the N-Terminal Region of DISC1. The N-terminal region possesses only two notable regions of conservation that correspond to a nuclear localization signal (NLS) sequence motif[18] and a serine-phenylalanine-rich (SF-rich) motif.[17] Another feature within the N-terminal region is the incidence of stretches of low-sequence complexity and compositional bias.[17] A dearth of predicted secondary structure elements is also evident.[3,16] This begs the question: is the N-terminal head domain compactly folded?

The N-terminus of DISC1 is often referred to as the globular head domain.[2,17] The term "globular" implies a folded 3-D structure in its native state. However, this region contains numerous low-complexity segments strewn throughout its sequence as identified by the program SEG.[19] Compositional bias involving excessive numbers of serine (consisting of 13−15% of residues), alanine, and glycine (together making up 16−23% of residues) residues was previously reported.[17] Typically, low-sequence complexity corresponds to disordered regions in proteins.[20] Protein disorder refers to segments or to whole proteins that fail to self-fold into fixed 3-D structure; such disorder often exists in its intrinsic state.[20]

To investigate this further, a secondary structure predictor coupled with a series of disorder predictors via a meta-predictor was applied to the 854 amino acid residues of full length human DISC1 (L-isoform, SwissProt Accession Number: Q9NRI5). These methods are capable of detecting secondary structure and disorder from sequence information alone, with a reasonable degree of accuracy [benchmarked ∼80% [21,22]]. The human DISC1 sequence was submitted to the disorder prediction meta-server, MetaPrDOS (meta Protein DisOrder prediction System),[23] that utilizes seven independent prediction methods to produce a consensus integrated prediction (Figure 1). Use of consensus disorder, based upon several methods, is a means of evaluating "high-probability" disorder regions by reducing the likelihood of an overprediction for the these regions. Consensus disorder employs a range of computational techniques including amino acid compositions, secondary structure prediction, and physicochemical properties. MetaPrDOS achieved higher prediction accuracy than all methods participating in the Critical Assessment for Techniques in Protein Structure Prediction 7.[23]

From the derived consensus disorder prediction, using a 5% false positive rate estimate threshold (the default setting), almost the entire N-terminus, ∼89% (310 out of 350 residues), is predicted to be disordered (Supporting Information Figure 1). Even with a more stringent false positive rate (2%), a significant fraction (228 out of 350 residues, ∼65%) is predicted to be disordered (Figure 1). These disordered residues occur in five major regions of greater than 25 continuous stretches of amino acid residues: (i) residue positions 1−35 (35 residues), (ii) 67−95 (29 residues), (iii) 146−205 (60 residues), (iv) 218−260 (43 residues), and (v) 278−322 (45 residues). A C-terminal region involving residue positions 710−741 was also predicted to be disordered. This result has consequences for experiments that require expressed protein. Extended regions of disorder should preferably be excised or shortened when creating genetic constructs for recombinant protein expression, purification, and attempts at crystallization.[21] Alternative strategies can be employed, such as use of fusion protein tags that increase protein solubility or foldability, and/or coexpression with partner proteins that overlap with regions of predicted disorder. Some regions do contain a majority of predicted consensus "order". For example, the regions spanning residue positions 36−66, 108−123, 130−145, 206−217, 261−277, and from residue position 333 onward the C-terminus were predicted to be ordered. Indeed, some of these regions correspond to predicted α-helices by PsiPred [24] (Figure 1). The first predicted coiled-coil region occurs only four amino acid residues beyond the α-helix located between 326 and 342, and follows a 45 residue disordered region that contains a low-complexity stretch of serine and glycine amino acid residues. There may thus be a case to revise the domain boundaries of the N-terminus to 1−326 (as opposed to 1−350). In further support of disorder, the PsiPred-predicted secondary structure results confirm that the N-terminus is largely devoid of confidently predicted secondary structure elements, and this region shows greater sequence variation among orthologs[17,25] than the C-terminal region (see conservation "heat map" in Figure 2). From inference, therefore, there is no evidence of a compact folded structure at the N-terminus of DISC1; protein disorder is more likely.

1.2. Protein Disorder in DISC1 is Potentially Important for Its Function. Disordered proteins can undergo a structural transition into a folded form upon binding to a target [21] and have several features that make them well suited to interact with

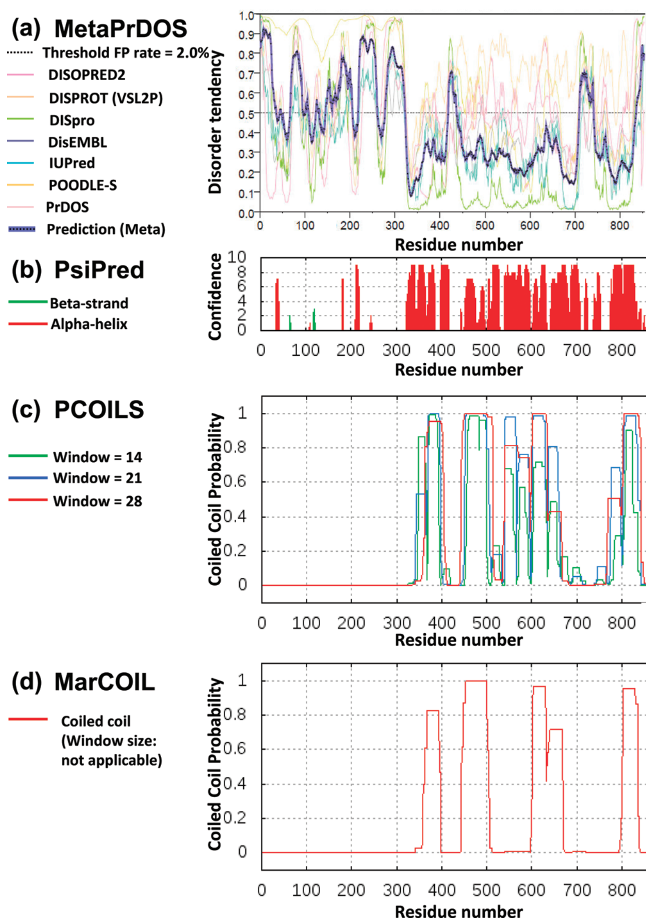

Figure 1. Structural overview of DISC1. Figure depicting application of a complementary suite of bioinformatics structure prediction methods on the sequence of human DISC1. These results are plotted in graphical format using an equivalent scale along the *x*-axis for residue position with the confidence or probability scores for each assignment along the *y*-axis. (a) MetaPrDOS, the "metaserver" that provides an integrated consensus disorder prediction (blue with dots) derived from seven independent disorder prediction methods. The individual prediction assignment for each method (refer to key) is also shown. (b) The PsiPred secondary structure prediction for human DISC1 (α-helices, red; β-strands, green) is provided. (c, d) Two independent coiled-coil method predictions applied to DISC1 is shown; PCOILS (input, multiple sequence alignment from Chubb et al.;[3] weight, yes; matrix, MTIDK; window, 14, 21, 28) and MarCOIL (input, human DISC1 sequence; matrix, MTIDK; TransProb, high; window, not applicable). From this figure, the dearth of secondary structure in the N-terminus of DISC1 is apparent. Various segments within the N-terminus overlap with assignments of consensus disorder tendency predictions (with a stringent false positive cutoff 2%). Also evident is the overlap between PsiPred predicted helices and the coiled-coil helices and also the occurrence of other regular α-helices within the C-terminus.

multiple targets. These proteins are characterized by a combination of low overall hydrophobicity and high net charge. Typically, they have significantly higher levels of certain amino acids, namely, Glu (E), Asp (D), Lys (K), Arg (R), Gly (G), Gln (Q), Ser (S), and Pro (P) (these constitute 60% of the N-terminal 350 amino acid residues in DISC1), and lower levels of others, specifically, Ile (I), Leu (L), Val (V), Trp (W), Phe (F), Tyr (Y), Cys (C), and Asn (N) (accounting for 21% of DISC1's N-terminal 350 amino acid residues).[20,21] Disordered proteins comprise a significant fraction of eukaryotic proteins; some estimates propose as many as 30% of eukaryotic proteins are either completely or partially disordered.[26]

Why have some proteins evolved to be disordered under physiological conditions? Various hypotheses have been proposed. A favored explanation is that the malleability of natively disordered proteins leads to advantages with respect to regulation and binding of diverse ligands. Another simple explanation suggests that disordered proteins form very large intermolecular interfaces within complexes.[21] Both hypotheses fit well with the current scenario for multiple DISC1 interactors. Over 200 protein interaction partners have been identified by yeast two-hybrid studies,[27−30] and a substantial number have been confirmed experimentally, as listed in the protein−protein interaction database IntAct.[31] Intrinsically disordered proteins often have some local and limited residual structure, which may be crucial to their interactions with their binding partners, as may be the case for the N-terminus of DISC1.

The importance of intrinsic disorder for the function of several individual "hub" proteins (i.e., proteins with a high degree of connectivity) has been studied in eukaryotic interactomes,[32] and its ability for transient reversible interactions reported[33] [and reviewed in refs 34 and 35]. It might be relevant that disorder within the N-terminus of DISC1, for example, aids in its dynamic reversible binding to cAMP-specific 3′,5′-cyclic phosphodiesterase 4B and 4D (PDE4B/PDE4D),[36] the contact points of which have been reported[37] (refer to Table 1); some of these regions correspond to predicted consensus disorder sites. An extended surface area for the N-terminus due to protein disorder combined with a potentially large surface area for the α-helical bundle/coiled-coil C-terminal region (see below) could accommodate multiple protein−protein interactions for DISC1.

1.3. Delineating the Coiled-Coil Regions within the C-Terminus and Assessing DISC1 Secondary Structure. The chief characteristic of most coiled-coils is a regular seven-residue sequence pattern known as the heptad repeat. These are often labeled from "abcdefg", where positions "a" and "d" are usually occupied by apolar residues that provide structural stability when two or more helices wind around each other to form "supercoils",[38] while the remaining positions are solvent exposed and usually provide surfaces for protein−protein interaction. These heptad repeats are readily identified by sequence analysis and structurally represent a distorted α-helix where the seven-residue repeat forms two turns (or 3.5 residues per turn) as opposed to a regular α-helix where 3.6 residues form one turn. Despite this apparent simplicity and relatedness at the sequence level, coiled-coils display a considerable degree of structural diversity; the helices may be arranged parallel or antiparallel and may form a variety of oligomeric states.[39] The secondary structure within DISC1 was analyzed and annotated using coiled-coil predictors along with the secondary structure predictor, PsiPred.[24]

Depending on which program is used for detecting coiled-coils e.g. CCHMM_PROF,[40] COILS,[41] MarCoil,[42] Multicoil,[43] PairCoil2 [44] and PCOILS,[45] the input provided (e.g., single sequence or multiple sequence alignment) and the stringency of the threshold parameters (e.g., use of a 14, 21, or 28 residue window-size, applying core position 'a' and 'd' residue weighting, and the matrix employed e.g. MTK, MTIDK, PDB) the precise number of coiled-coil regions, their extent and boundaries within DISC1, vary.[3,16,17,46] However, all the aforementioned programs predict coiled-coil regions within the C-terminus by broad consensus (Supporting Information Figure 2). By considering the output

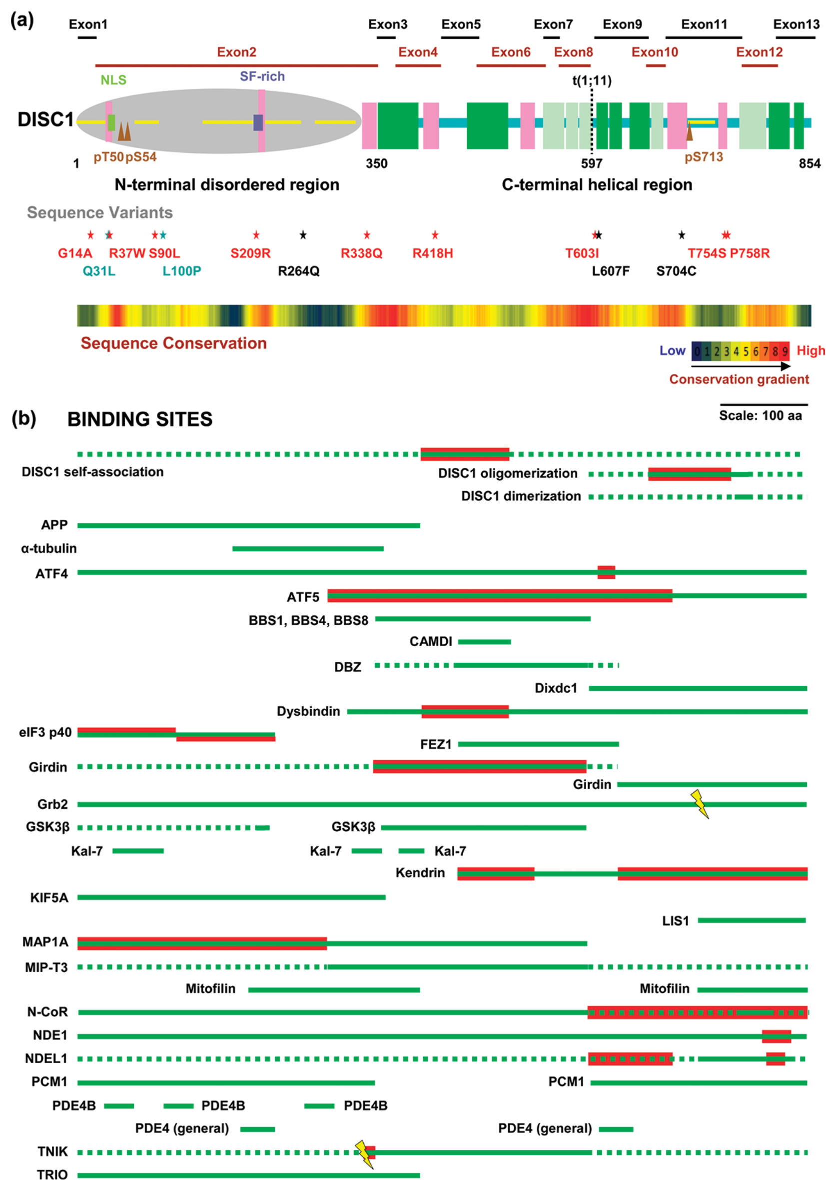

Figure 2. DISC1 structure schematic, sequence variants and binding sites. (a) Schematic depicting mapped secondary structure (pink, regular α-helices; green, coiled-coil helices; light-green, ambiguous helix, maybe regular or coiled-coil) and extended consensus disorder predictions (thick yellow lines) from Figure 1, drawn to scale. Position of sequence motifs and features are highlighted; these include the nuclear localization signal (NLS), serine-phenylalanine-rich (SF-rich) motif, phosphorylation sites [pT50 (putative), pS54, pS713, brown triangles][14] and translocation break point t(1;11) at amino acid position 597.[1,2] Known mouse missense mutations (blue star), human common variants (black star), and human ultrarare mutations (red star) are indicated. Ortholog sequence conservation is shown mapped below the DISC1 schematic to represent the strength of conservation for each sequence position in DISC1 (gaps excluded); these were calculated using the program AL2CO[194] using the multiple sequence alignment from Chubb et al.,[3] but excluding the fragment sequence of *Bos taurus* that lacked the N-terminal region to avoid bias in calculation. The AL2CO conservation score was averaged over a 31 amino acid sliding window (15 residues either side of a central residue) and colored accordingly (refer scale for conservation gradient). The conservation "heat map" brings out the greater variation in the N-terminus versus the C-terminus. Apparent from this depiction are the highly conserved NLS and SF-rich motifs. Interestingly, the predicted regions of disorder map to areas of least conservation. The 13 exons that encompass full-length DISC1 are shown above the schematic. (b) Known regions of DISC1 involved in self-association and protein−protein interactions determined using truncated protein constructs are shown. Green lines indicate constructs which are sufficient for protein binding and thus include a protein binding domain. Where two or more overlapping constructs are each sufficient for interaction, the overlapping region is indicated with a solid line and the remainder with dotted lines. Red boxes indicate regions which abolish or severely impair protein binding if deleted; these may represent protein binding domains or other essential structural features. Yellow lightning bolts indicate small groups of 2−4 amino acid residues, which if mutated, abolish or reduce protein binding. Data derived from Table 1; see references therein. All lines are drawn to scale with the DISC1 structure schematic in (a).

from two of the better performing methods,[47] at least four regions possess coiled-coil potential by consensus (Figure 1). These correspond to residues 347−393, 452−499, 603−680, and 805−828. Some of these regions contain stretches of consecutive heptad repeats (e.g., residues 462−496 are predicted to contain five consecutive heptad repeats[16]), while others possess short "breaks" in their heptad repeat registers. There also exists some ambiguity in assignment of coiled-coils. For

example, within a predicted stretch of α-helices (between 540 and 600), the program PCOILS[45] suggests that the third coiled-coil region begins earlier at around position 540, while MarCOIL[42] suggests a later start position around 603. Two leucine zipper motifs (i.e., where leucine residues are present at position "d" of coiled-coil heptad repeats), 458−486 and 607−628, are identified by PROSITE,[48] while a third, 808−829, was suggested by manual examination.[46] The PsiPred secondary structure prediction of α-helices in the C-terminus overlays appropriately with the regions of coiled-coil helix propensity (Figure 1), but also suggests a number of other "regular" α-helices that otherwise lack the heptad repeat (Figure 2). Some examples include confidently assigned α-helices that occur at positions 326−342, 400−416, and 686−708. There appear to be minor differences in agreement between the PsiPred version used by the PCOILS server (Figure 1) and the PsiPred server version 3.0 (http://bioinf.cs.ucl.ac.uk/psipred/). In this case, for purposes of the schematic in Figure 2, only the consensus regions between the two predictions are indicated.

To summarize, by consensus the C-terminal region is predicted to be made up of at least four regions of coiled-coils that contain identifiable heptad repeat registers, and at least five "regular" α-helices that do not possess heptad repeats interspersed along the sequence of DISC1. The predicted coiled-coil segments in DISC1 are discontinuous and more dispersed than, for example, those that occur in myosin, kinesin, or keratin that contain continuous stretches of greater than 100 amino acids with coiled-coil propensity. Interestingly, no β-strands are predicted within the C-terminus. Overall, therefore, the C-terminal region appears to possess the characteristics of a series of helical bundles that coexist with, or partly engage in, coiled-coil interactions.

1.4. DISC1 Self-Association, Oligomeric State, and Aggregation. The difficulty in expressing and purifying folded full-length DISC1 protein has been a major stumbling block in the DISC1 field. Previous work tapping structural aspects of the protein has relied on truncated constructs. These however have been limited to the C-terminus, which is unsurprising given the disorder prediction for large swathes of the N-terminal region of DISC1.

Brandon et al.[49] noted that DISC1 formed large (≥250 kDa) species that they believed to be DISC1 dimers, or higher order structure and quoted unpublished data that multimerization of DISC1 could be demonstrated through interaction of FLAG- and MYC-tagged DISC1 in HEK293 cells. A region spanning residues 403−504 of DISC1 was later shown to be a functional self-association domain by pull-down experiments with deletion mutants expressed in HEK293 cells[50] (Figure 2). This region includes a predicted leucine zipper motif at positions 455−495, a motif that often promotes higher order oligomeric states. A wealth of biochemical and biophysical data for several purified C-terminal fragments was published by three recent studies.[15,16,51] These C-terminal constructs included regions in DISC1 spanning amino acid residues 316−854, 598−785, 598−854, 640−854, 640−854Δ22 (i.e., minus 748−769), 668−854Δ22 (i.e., minus 748−769), 668−854, and 765−854, and the results are summarized below.

In the first study, Leliveld et al.[15] used size exclusion chromatography (SEC) to characterize multimerization of the recombinant DISC1 fragment 598−854. DISC1(598−854) was found to exist as dimeric, octameric, and multimeric species under physiologically relevant conditions. The dimer displayed a comparable far ultraviolet circular dichroism (CD) spectrum as the combined oligomer/multimer fraction, indicating that DISC1-(598−854) self-associated as folded subunits. The DISC1-(598−854) construct gradually formed aggregates (when stored at 80 μM), suggesting a self-association continuum that paralleled aggregation. Interestingly, the authors noted that only the octameric state of the DISC1(598−854) construct, but not the dimers or multimers, was required for functional interaction with nuclear distribution protein E homologue like-1 (NDEL1) indicating that an oligomerization optimum was necessary for interaction between the two proteins. A subsequent study using another construct, DISC1(640−854), confirmed that this oligomeric optimum was necessary for NDEL1 interaction.[16] Insight into the size of a longer construct that encompassed all putative coiled-coil regions, that is, DISC1(316−854), was obtained by dynamic light scattering (DLS) experiments. DISC1(316−854) had a hydrodynamic diameter (D_h) measurement of 15 ± 1 nm. Additionally, a DLS reading for the DISC1(316−854):NDEL1 (1:1) interaction gave a D_h of 19.2 ± 1 nm. The authors also noted that NDEL1 did not bind aggregates of full length DISC1 in vivo.

In the second study, using a range of other purified constructs, Leliveld et al.[16] undertook SEC and covalent cross-linking and showed that both DISC1(668−854) and DISC1(640−854) formed a mixture of dimers, one or two types of oligomer, and soluble multimers. Although the DISC1(640−854) dimer could be isolated as a stable species, part of the oligomers immediately dissociated to dimers after fractionation and the isolated dimers did not associate into oligomers at the same speed. This behavior was illustrated by DLS readings, where the DISC1(640−854) dimer had a D_h of 6.2 ± 0.8 nm and the combined oligomer/multimer fraction had a D_h of 7.8 ± 0.4 nm, just like the non-fractionated sample. CD spectroscopy of the DISC1(640−854) dimer fraction and the combined oligomer/multimer fraction showed that both contained the same overall mixed alpha/beta fold (after deconvolution of CD spectra: 32% α-helix, 18% β-strand, 21% turnlike, 30% loop). Unlike the DISC1(598−854) construct used in the previous study,[15] DISC1(640−854) did not have an aggregation threshold, rather the size distribution shifted toward oligomers and dimers upon concentration. Size exclusion chromatography of DISC1(765−854) showed that it only formed an ∼35 kDa species, consistent with a covalent cross-linking experiment that showed this complex to be dimeric. Circular dichroism spectroscopy showed DISC1(765−854) to be mainly α-helical, consistent with the presence of a predicted coiled-coil region. SEC analysis and native polyacrylamide gel electrophoresis of DISC1(598−785) mainly showed high molecular weight multimers and small amounts of dimers and oligomers. Its CD spectrum however was comparable to those of both DISC1(668−854) and DISC1(640−854), suggesting that overall folding in this case was not affected by truncation. Laddering of DISC1(640−854) and DISC1(668−854) indicated regular oligomerization that was higher and less regular for DISC1(598−785), but absent for DISC1(765−854). These findings suggested that dimerization encoded within the last coiled-coil domain (note, Leliveld et al.[16] partitioned the C-terminal region into four coiled-coil domains) was necessary for an orderly, concerted oligomerization together with region 668−747. SEC analysis for both DISC1(640−854) and DISC1(668−854), and another construct DISC1(640−854)Δ22 (minus 748−769), showed that the common disease-associated C704 variant (refer to section 3) had, on average, a moderately higher fraction of oligomers than S704. CD spectroscopy of both variants, however, yielded exactly the same results. These results

indicated that the fourth coiled-coil domain (765−854) formed a stable dimer and that the region between residues 668−747 was responsible for oligomerization. Although the third coiled-coil domain was capable of aiding the formation of higher order oligomers and multimers, it was dispensable for oligomerization of the whole C-terminal domain. The authors concluded that the oligomerization domain, if indeed folded like a coiled-coil domain, was more likely to be involved in homomeric intermolecular interactions and that the S704C variant modulated this. They further suggested that a likely unstructured subdomain within 668−774 controlled oligomerization. Indeed, this region overlaps with the only extended (>25 amino acid) consensus disorder prediction in the C-terminus that encompass residues 710−741 (Figure 2).

Leliveld et al. demonstrated that detergent-insoluble DISC1 could be seen by immunoreactivity in ~20% of post-mortem brains of patients with chronic mental diseases, but not in healthy controls.[15] DISC1 aggregates did not bind NDEL1, whereas insoluble DISC1(316−854) aggregates did copurify with dysbindin, the product of another prime candidate gene associated with mental illness,[52] in brains of a subset of patients with mental illness, but not in healthy controls.[51] These findings highlight the potential of DISC1 protein aggregation and recruitment of binding partners as a biological mechanism in a subset of mental illness cases,[5] and while the current study only analyzed post-mortem brain tissue, it highlights the possibility of DISC1 being a useful biomarker in future for diagnosis, particularly if these results were also noted in more accessible tissues.

The biophysical studies undertaken to date have provided much needed insight into the C-terminal portion of DISC1. However, many questions remain and some new questions arise. How many quaternary states does full-length DISC1 adopt? How many more self-association regions remain to be mapped? Do these self-association regions act in concert in the assembly of full-length DISC1? Or do they fulfill different functions? Are DISC1 protein−protein interactions dependent on quaternary state? Are the experiments that used truncated constructs physiologically relevant in context of the full length protein? Depending on the conditions and the C-terminal construct examined, the oligomeric state of DISC1 is clearly delicate[16] and examples exist where truncated constructs affect the oligomerization state of coiled-coil domains.[53−56]

1.5. The Molecular Weights of DISC1 Species. Over 40 differentially spliced transcripts of *DISC1* have been identified from human brain tissue to date.[57] It is not yet known how many are translated, but it is clear that there are multiple DISC1 protein species identified by Western blotting using different antibodies raised against DISC1 or fragments thereof. The most commonly described molecular weights of DISC1 are around 100 kDa (generally presumed to correspond to a protein translated from the canonical 13 exons of the *DISC1* gene,[58] known as the long or L variant; Figure 2) and one or more protein species of approximately 75 kDa. Figure 3 displays the sizes of various DISC1 species identified to date, with the predicted molecular weights of proteins derived from known transcripts shown for comparison. It should be noted that because molecular weights displayed are as quoted by the original authors, species of similar sizes may, in fact, represent a single species, the size of which has been variably estimated. Conversely, multiple protein species may share the same apparent size; for example, a 75 kDa DISC1 protein can be detected using antibodies raised against either the N- or C-terminal regions of DISC1, implying the existence of distinct "n-75" and "c-75" species.[59]

Apparent discrepancies between molecular weights of DISC1 species predicted from transcripts and those seen in vivo could potentially be explained by multiple causes. First, post-translational modifications such as phosphorylation[14] may raise the molecular weight of the protein. Alternatively, if DISC1 forms an elongated structure, as could occur through its putative coiled-coil regions, then this would cause DISC1 to migrate slower through a gel than a similarly sized globular protein would and, therefore, show a higher apparent molecular weight. It is also possible that some species are generated not by alternative splicing but by post-translational cleavage of longer protein species as hinted at by the apparent generation of both ~100 and ~75 kDa species by a construct exogenously expressing the DISC1 L variant in HEK293 cells.[60] The larger (130 kDa and higher) DISC1 species can be hypothesized to arise through DISC1 forming high binding affinity protein complexes,[49] either as DISC1 oligomers or with protein binding partners, that remain intact under standard experimental conditions.

Variation in DISC1 species detected by different antibodies can reasonably be expected if they are raised against epitopes found in some but not all DISC1 variants, or are expressed in a tissue or cell type restricted fashion. Nevertheless, the presence of such a diverse array of protein species and variation between antibodies,[61] combined with uncertainty as to how splice variants correspond with protein species and the lack of an established "gold standard" antibody or DISC1 knock-down technique to aid characterization, raises the serious possibility that some of the apparent DISC1 species represent nonspecific antibody binding events. This problem has been recognized but not yet resolved and remains a priority for the field.[61] Systematic DISC1 isoform analysis, whether by generation of exon selective and specific antibodies or through purification and mass spectroscopic identification of individual DISC1 species, is required if we are to understand this fundamental aspect of DISC1 function.

2. DISC1−PROTEIN INTERACTION SITES

DISC1 acts as a protein scaffold, binding over 200 interactors, many of which were originally identified in yeast two-hybrid experiments[27−30] but have now been confirmed in mammalian models, for example, in cell lines or primary tissue lysate. Interestingly, some of the DISC1-interactors also interact with one another, suggesting their involvement in common pathways (see ref 4 for a recent review). More recently, DISC1 has been linked via convergent pathway studies to Huntington's disease,[62] suggesting a potential common biological underpinning with respect to shared aspects of cognitive, psychotic, and mood deficits between these otherwise distinct disorders, further enhancing its importance as a pathfinder in neuropathology.

For many of these interactors, truncation mutants of DISC1 have been used to elucidate putative sites of protein−protein interaction. In general, these fall into two categories. First, demonstration of DISC1 truncates that retain protein-binding capability; these can be assumed to contain the minimum binding site for the interaction partner. Second, demonstration that deletion of certain regions of DISC1 abolishes protein−protein interaction; these demonstrate that the region is critical for interaction. However, it may not necessarily reflect a protein binding site, but instead represent important DISC1 structural features, such as oligomerization sites or subcellular localization signals that are crucial for protein−protein interaction. These experiments are described in Table 1 and summarized schematically in Figure 2

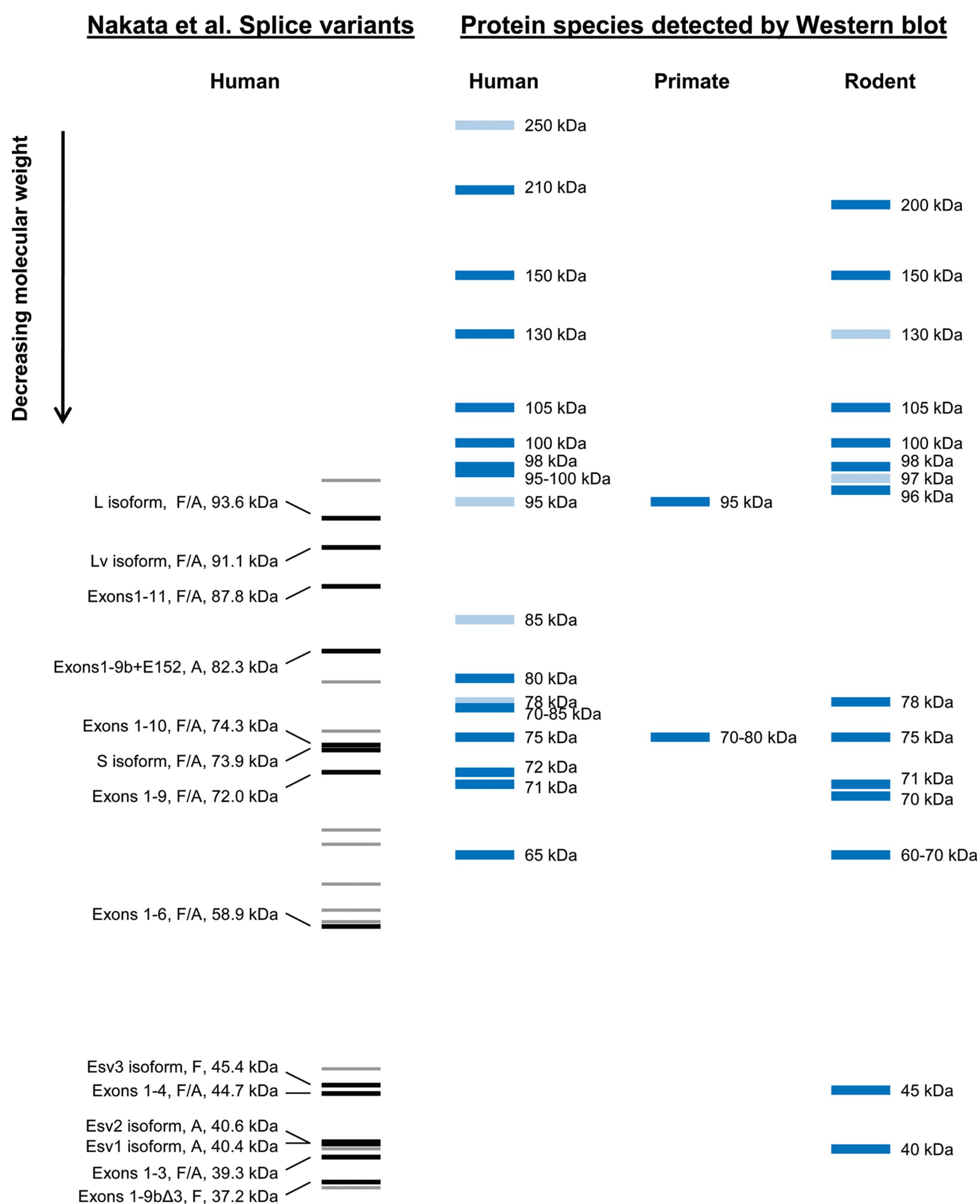

Figure 3. DISC1 transcript splice variants and protein species. The sizes of predicted DISC1 proteins from known transcripts with protein species detected by Western blotting using anti-DISC1 antibodies are compared. First column: Black lines indicate predicted sizes of proteins encoded by each of the splice variants described by Nakata et al.[57] that were found in at least 30% of either human adult (A) or fetal (F) hippocampal samples examined. Refer to original paper for details of exon structure for each variant. Gray lines indicate the predicted sizes of variants found in a lower proportion of samples. Two short variants (22.2 kDa and 2.5 kDa, each found in single samples) are not shown. Remaining columns: Endogenous DISC1 protein species detected in human (second column), primate (third column), or rodent (fourth column) by Western blotting. Dark blue lines indicate species detected in brain tissue, and pale blue lines represent species described to date in cell lines only. Sizes indicated are as described by authors of the original papers. Where no size was estimated, protein is not shown. Notably, Ishizuka et al. also saw several 100−150 kDa species in mouse brain using a range of antibodies,[61] and Leliveld et al. detected several short (50 kDa and lower) isoforms in human brain using antibodies against the DISC1 C-terminus.[15] Human Western blot data taken from refs 15, 49, 59, 64, and 114, primate data from ref 25, and rodent data from refs 8, 59, 60, 80, 140, and 195−199.

(refer to Supporting Information Table 1 for DISC1-interactors that have had regions mapped by yeast two-hybrid experiments only).

It is noteworthy that many of binding partners listed in Table 1, like DISC1, also contain or are predicted to contain coiled-coil domains. It would be interesting to find out how many of these proteins utilize their coiled-coil domains to facilitate DISC1-interaction; such interactions may reflect important structural roles. Some of the interactors in Table 1 are homologous or paralogous proteins (e.g., the centrosomal and microtubule-organizing proteins NDE1/NDEL1; cAMP-degrading phosphodiesterases PDE4B and PDE4D; activating transcription factors ATF4 and

Table 1. Regions of DISC1 Important for Interaction with Its Protein-Binding Partners[a]

protein	assay type	interacting DISC1 constructs	noninteracting DISC1 constructs	refs
APP	OE, BL	1−854 (L isoform), 1−402, Lv, S, and Es isoforms	403−854*	63
α-tubulin	OE, BL	181−357	1−181, 358−598, 598−854	49
ATF4	OE, RP	1−854	1−854Δ(607−628), 1−854Δ(Exon9)	64
ATF5	OE	1−854, 293−854	1−587, 697−854*	27
BBS1, 4 and 8	OE	1−854, 349−600	1−348, 601−854	65
CAMDI	OE, BL	446−507	340−400*, 533−590	66
DBZ	OE, CL, BL	348−597, 446−633	598−854	67
DISC1 (self-association)	OE, RP	1−854, 1−504, 1−536, 1−597, 402−854	1−402, 1−854Δ(403−504)	50
DISC1 (dimers)	RP	598−785, 640−854, 640−854Δ(748−769), 668−854, 765−854		16
DISC1 (oligomers)	RP	598−785, 640−854, 640−854Δ(748−769), 668−854	765−854	16
Dixdc1	OE, BL	1−852, 596−852 (mouse)	1−220, 221−355, 356−595* (mouse)	68
Dysbindin	OE, RP, post-mortem brain[#]	316−854, 316−854Δ(403−504)		51
eIF3 p40	OE, CL, RP	1−854, 1−231, 1−347	1−115, 116−231, 116−347, 232−347, 349−854	69
FEZ1	OE	1−854, 446−633	1−854Δ(446−533)	60
Girdin[&]	OE, RP, PN, BL	1−854, 1−361, 1−597, 1−633, 1−832, 347−633, 347−854, 633−854	1−347, 361−832, 598−832	70, 71
Grb2	RP, BL	1−854	1−854(mut730/731)	72
GSK3β	RP, BL	1−220, 195−238, 211−225, 356−595 (mouse)	221−355, 596−852, other peptides within: 195−238 (mouse)	11
Kal-7	OE, PA, PN, BL	1−853, 1−401, peptides:, 41−100, 321−355, 376−410	1−347, 290−853, 402−853, 1−853Δ(350−394), other peptides	73
Kendrin	OE	1−854, 446−854	1−854Δ(446−533), 348−597, 446−533, 446−633, 598−854	74, 75
KIF5A	OE, CL, RP, BL	1−832, 1−361, 1−597	362−832, 598−832	72, 76
LIS1	OE, CL	1−854, 727−854 (data not shown)	1−597	49, 50
MAP1A	OE	1−854, 1−597	293−854	27
MIP-T3	OE	1−854, 1−597, 293−854	687−854	27
Mitofilin	OE, RP, CL, PN, BL	200−400, 598−854, 726−854	358−597*, 598−726	77
N-CoR	OE	1−854, 1−854Δ(Exon9), 1−854Δ(Exon9−11), 1−854Δ(Exon13)	1−854Δ(Exon9−13)	64
NDE1	OE, CL, BL	1−854	1−854Δ(802−835)	78
NDEL1[&]	OE, CL, RP, BL	1−854, 1−835, 293−854, 598−854, 727−854	1−357, 1−597*, 1−597Δ(403−504), 1−697*, 1−726*, 1−738*, 1−801*, 598−854Δ(807−828), 697−854	27, 49, 79, 80
PCM1	OE	1−854, 1−854Δ(802−835), 1−348, 601−854	349−600	65
PDE4B1 and 2	OE, CL, PA	peptides: 31−65, 101−135, 196−225, 266−290, 611−650	other peptides	36, 37
PDE4B3	OE, CL, PA	1−854, 1−250, 1−300, 48−854, 124−854, 190−854, 220−283, 220−854, 359−854	1−180, 284−854	36, 37
PDE4D3	OE, CL, PA	Peptides: 191−230, 611−650	other peptides	36, 37
TNIK	OE, BL	1−854, 1−597, 1−697, 1−726, 1−748, 1−801, 1−835, 46−854, 150−854, 245−854, 291−854, 302−854, 313−854, 324−854, 335−854	1−854(mut 336−338, 344), 348−854*, 403−854	81
TRIO	OE, RP	1−852, 1−408 (mouse)	401−645, 636−852 (mouse)	82

[a] DISC1 truncation mutants, recombinant proteins, and peptides which have been tested for protein−protein interaction beyond yeast two-hybrid screening are listed, with outcome and corresponding references. Refer to Abbreviations for full names and aliases of proteins listed in Table 1. Assay type abbreviations: BL = Coimmunoprecipitation in rodent brain lysate; CL = coimmunoprecipitation in cell line; OE = coimmunoprecipitation of overexpressed protein; PA = interaction on an in vitro peptide array; PN = coimmunoprecipitation in rodent primary neurons; RP = interaction of recombinant protein in vitro (pull down assay or copurification); [#] = in a subset only, specifically with aggregated DISC1. Asterisks (*) denote constructs which showed some interaction, but for which this was dramatically reduced compared to other DISC1 constructs. "Peptides" denote regions of DISC1 for which a variety of short peptides, spanning the listed region were tested. "Mut" denotes that mutation of the listed amino acids was sufficient to block interaction. For DISC1-DISC1 interaction sites, "self-association" represents regions shown to bind in cell models, while "dimers" and "oligomers" represent oligomeric states of the purified constructs. Where mouse constructs were used, these are mentioned. Where specific human DISC1 alternatively spliced isoforms were tested, these are indicated long (L isoform, 1−854 amino acids), long variant (Lv isoform), short (S isoform), and extremely short (Es isoform). The potential binding protein region summary is summarized schematically in Figure 2 drawn to scale with DISC1. Ampersands (&) denote discrepancies in noted interaction domains with DISC1; these are discussed in more detail in the text.

ATF5; Rho guanine nucleotide exchange factors Kalirin-7 and TRIO; see Abbreviations for full definitions). These pairs or families of proteins may thus possess equivalent binding sites with respect to DISC1, and it would be worthwhile to tease apart common and variable binding regions. For example, from peptide array interaction studies of PDE4B and PDE4D with DISC1, it was noted that the PDE4 family possessed both common binding sites and also isoform-specific ones (Table 1).[37]

In two instances, different groups have reported seemingly contradictory DISC1 binding domains for the same protein. This was the case with girdin, also known as KIAA1212.[70,71] This discrepancy can however be partially explained by the data of Kim et al.,[70] which implies the existence of two distinct girdin-contact sites on DISC1, one in the central region (347−633) and the other at the C-terminus (633−854). It can therefore be presumed that Enomoto et al.[71] detected the central binding domain only. The only major inconsistency between the two data sets is that Enomoto et al. did not see girdin interaction with their C-terminal construct (597−854). One possible explanation for this is that the large GST-tag on the construct of Enomoto et al.[71] may have interfered with the binding process in a way that the smaller HA-tag employed by Kim et al.[70] did not. Alternatively, the different N-terminal cut-off points utilized may have contrasting effects on DISC1 coiled-coil formation and therefore alter its tertiary structure, changing its binding properties.

Two binding domains for NDEL1 on DISC1 have also been suggested, one at the extreme C-terminus[49,79] and the other central.[27] Crucially, amino acids 727−854 have been shown to be sufficient for NDEL1 interaction, while the binding site in the center of DISC1 was implicated by deletion studies only. It therefore seems more likely that NDEL1 binds DISC1 at the C-terminus, but that some structural feature in the center of DISC1 is important for the interaction. The most plausible candidate would be the DISC1 self-association domain reported by Kamiya et al.,[50] given that NDEL1 interaction has been shown by Leliveld et al.[15] to be dependent on the oligomeric state of DISC1.

Instances such as those for girdin and NDEL1 are invaluable, as they serve to illustrate the need to carefully consider both direct and indirect effects of deletion constructs on DISC1 structure and their capacity for protein−protein interaction. They point also to the value of high resolution interaction domain mapping by peptide arrays and/or site-directed mutagenesis to test the effects of specific amino acid substitutions or post-translational modifications, including clinical mutations and natural polymorphisms.

3. DISC1 PROTEIN SEQUENCE VARIATION: STRUCTURAL AND FUNCTIONAL IMPACT

Apart from the original translocation mutation,[2] and a C-terminal frame-shift four nucleotide deletion in *DISC1*,[83] both of which have the potential to encode protein truncations, numerous nonsynonymous common variants and ultrarare missense mutations in DISC1 have been associated with major mental illness. Noncoding variants in *DISC1* that have also been associated with mental illness are reviewed elsewhere.[3,4] Here, we assess the protein coding variants that have been associated with major mental illness. We map their location and assess their predicted consequences on structure and function with regard to sequence conservation, location on secondary structure, and overlap with known binding sites and other known or predicted motifs (Table 2).

3.1. Human Common DISC1 Sequence Variants. To date, three common amino acid variants in the *DISC1* gene have been identified as associated with psychiatric illness: R264Q, L607F, and S704C (reviewed in refs 3 and 4). While the studies addressing the consequences of R264Q have to our knowledge been limited to a modest effect on cortical thickness in the lateral occipital gyrus,[94] there is a growing body of biological evidence for the causative effects of L607F and S704C. Other common variants have also been described, but they do not appear to be significantly associated with schizophrenia or bipolar disorder.[87,89]

L607F. Carriers of the F607 allele have been shown to display reduced gray matter in the superior frontal gyrus, anterior cingulate cortex, and left supramarginal gyrus compared to L607 homozygotes, as well as to suffer from more severe positive symptoms of schizophrenia in the case of patient carriers and show increased dorsolateral prefrontal cortex activity during a working memory task.[94,95] The biological mechanisms underlying this remain to be elucidated; however, several observations provide clues. Specifically, in cell culture models, the F607 variant is associated with reduced noradrenaline release, lower levels of the DISC1-interacting protein PCM1 (pericentriolar material 1) at the centrosome, as well as mitochondrial trafficking defects.[96,97] Curiously, carriers of the F607 allele also express lower levels of a *DISC1* specific transcript whose expression is altered in schizophrenia patients.[57]

S704C. The role of S704/C704 in brain structure has received considerable attention, with the C704 allele being associated with increased volumes of several brain regions.[98,99] Variation of amino acid 704 also appears to affect gray matter volume, including parahippocampal formations,[98,100,101] as well as affecting white matter integrity.[102] Functional imaging studies have shown S704 homozygotes to have decreased hippocampal activation during memory tests, greater hippocampal and paracampal activation during memory encoding, and greater prefrontal cortex activation during verbal fluency tests compared to carriers of C704.[100,101,103] The C704 allele has also been associated with higher memory test scores, lower cognitive ability scores in elderly men, and more severe positive schizophrenia symptoms in patients.[100,104,105] At the molecular level, like L607/F607, variation at amino acid 704 is implicated in altered PCM1 localization and schizophrenia-related *DISC1* transcript expression.[57,106] Additionally, the C704 allele is associated with reduced activity of the kinases extracellular signal-regulated kinase 1 (ERK1) and RAC-alpha serine/threonine-protein kinase (AKT), altered binding affinities of DISC1 for NDE1 and NDEL1, and variation in DISC1 oligomeric status.[16,78,79,98]

3.2. Human Ultrarare DISC1 Mutations. The first large-scale case-control mutation study of *DISC1* was performed by Song et al.[87] who sequenced the coding exons and splice junctions of *DISC1* in 288 patients with schizophrenia and in 288 controls. Six patients with schizophrenia were heterozygous for ultrarare missense variants not found in the 288 controls (p = 0.015) and shown to be "ultrarare" by their absence in a pool of 10 000 unrelated control alleles. They inferred that ultrarare structural variants in DISC1 are associated with an attributed risk of 2% for schizophrenia. In addition, they also confirmed that two of the three aforementioned common variants (Q264R and S704C) elevate the risk for schizophrenia slightly (odds ratio 1.3, 95% confidence interval: 1.0−1.7). The five identified novel ultrarare

Table 2. Known or Predicted Consequences of DISC1 Protein Sequence Variation on Structure and Function[a]

sequence variant in DISC1	sequence conservation and location on structure	experimental effects where known and potential binding region overlap	notes, motifs, and potential effects on structure and function
		Human common variants	
R264Q (Q264 minor allele frequency 0.2599)	R in *hs*, *pt*, *ma*; not well conserved, Q not seen in any ortholog; loop	Binding region: APP, α-tubulin, KIF5A, MAP1A, mitofilin, PCM1, TRIO	Lies close to peptide region in DISC1 266−290 mapped as PDE4B1 binding. Loss or gain of charge alters interaction propensity?
L607F (F607 minor allele frequency 0.0986)	*hs, pt, ma, bt, cf, rn, mo, tr, dr*; strictly conserved; coiled-coil helix	Experimental: Lower levels of the DISC1-interacting protein PCM1 at the centrosome for F607 (see main text for other details of effects of L607F). Binding region: ATF4, ATF5, Dixdc1, FEZ1, N-CoR, PCM1.	Located directly within a leucine zipper motif, within a heptad repeat at position "d". Change to Phe could alter the intricate Leu-Leu packing between adjacent helices. The presence of repeating leucine residues at position "d" in leucine zippers is critical for modulating coiled-coil stability, maintaining oligomeric state, partner selection, and orientation of coiled-coil helices as shown by numerous substitution experiments by Harbury and co-workers on the GCN4-zipper for its leucine positions, with other hydrophobic residues.[84,85] L607F likely to impact on the structure of the protein.
S704C (C704 minor allele frequency 0.2472)	*hs, pt, ma, bt, rn, mo*; largely conserved amino acid position: G in *cf, tr, dr*; α-helix	Experimental: S704C alters NDE1, NDEL1 interaction. Structurally, C704 has on average, a moderately higher fraction of oligomers than S704. C704S implicated in altered PCM1 localization (see main text for other details of effects of S704C) Binding region: DISC1 (oligomerization), Dixdc1, girdin, kendrin, N-CoR, PCM1.	S704C lies close (context residue) to a predicted nuclear export signal (NES).[86] The context around the leucine residues in the nuclear export signal that mediates interaction has been suggested to play an important role by la Cour et al.[86] (see section 4).
		Human ultrarare mutations seen in cases only	
G14A[87]	*hs, pt, cf*; not conserved; disordered (loop)	Binding region: APP, eIF3 p40, KIF5A, MAP1A, PCM1, TRIO.	Lies within region that is Gly and Ala rich (low complexity).
R37W[87]	*hs, pt, ma, cf, rn, mo, tr, dr*; strictly conserved; α-helix	Experimental: Preliminary results suggest W37 increases DISC1 localization to mitochondria.[88] Binding region: APP, eIF3 p40, KIF5A, MAP1A, PCM1, PDE4B, TRIO.	Proximal residue in mouse Q31L mutation (equivalent to human R35) causes reduced binding to PDE4B1. Hence, R37W might also affect PDE4B binding, (since it lies within binding peptide mapped to 31−65). It also lies close to Kal-7 binding site (41−100). R37W lies within tetra-arginine nuclear localization signal motif (R35-R38).[64]
S90L[87]	*hs, pt, cf*; not conserved; disordered (loop)	Binding region: APP, eIF3 p40, Kal-7, KIF5A, MAP1A, PCM1, TRIO.	L90 less favorable at this position, but not well conserved.
S209R[89]	*hs, pt, ma, cf, mo, tr, dr*; very highly conserved; α-helix	Binding region: APP, α-tubulin, eIF3 p40, KIF5A, MAP1A, mitofilin, PCM1, PDE4 (general), TRIO.	Within conserved SF-rich motif, within the PDE4 family binding region (191−230) and close to GSK3β binding region (211−225). Predicted phosphorylated site by NetPhosK[90] as a cdc2 kinase target site and also adjacent predicted phosphorylated sites[90,91] T207 and S211.
R338Q[89]	*hs, pt, ma*; Q in orthologs *rn, mo, tr, dr*; α-helix	Binding region: APP, α-tubulin, ATF5, Kal-7, KIF5A, MIP-T3, mitofilin, PCM1, TNIK, TRIO.	Wang et al.[81] found that alanine substitutions for V336, L337 and R338 together with N344 resulted in a weakened DISC1 interaction with TNIK. R338Q may thus be indirectly implicated as functionally important. However, because this position is occupied by Gln in some nonprimate orthologs, it would be useful to undertake further mutagenesis validation using the loss-of-charge variant. R338Q also lies within the defined Kal-7 binding region (321−355).

Table 2. Continued

Human ultrarare mutations seen in cases only			
R418H[87,89]	*hs, pt, ma, tr, dr*; present as H in *cf*; end of regular α-helix	Binding region: ATF5, BBS1, BBS4, BBS8, dysbindin, DISC1 (self-association), girdin, GSK3β, MIP-T3.	Located within GSK3β defined binding region (356−595) and near Kal-7 binding region (376−410).
T603I[87]	*hs, pt, ma, bt, cf, rn, mo*; highly conserved (S in *tr, dr*); coiled-coil helix	Binding region: ATF5, Dixdc1, FEZ1, N-CoR, PCM1.	Potential to disrupt phosphorylation (predicted phosphorylation site by NetPhos[91]). Also close to ATF4 binding region 606−628 and near PDE4 (general) binding region 611−650.
T754S[89,92]	*hs, rn, mo*; end of regular α-helix	Binding region: Dixdc1, girdin, kendrin, LIS1, mitofilin, N-CoR, PCM1.	Conservative substitution with variant T754S, but position not well conserved.
P758R[92]	*hs, pt, ma, bt, cf*; loop	Binding region: Dixdc1, girdin, kendrin, LIS1, mitofilin, N-CoR, PCM1.	Structurally unfavorable change within loop.
Mouse missense mutations			
Q31L[8]	*mo, rn*; Q31 is not conserved; in humans equivalent amino acid R35; boundary of loop and α-helix	Experimental: Reduces PDE4 activity, but causes an increase in GSK3 enzymatic activity. Q31L reduces interaction with both PDE4B and GSK3 (see main text for more details of effects of Q31L) Binding region: APP, eIF3 p40, KIF5A, MAP1A, PCM1, PDE4B, TRIO.	Part of peptide region in DISC1 31−65 mapped as important for PDE4B1 binding. However, outside known GSK3β-binding regions. These have however been incompletely mapped. Clapcote et al.[8] suggested likely effects of the mice mutations based upon the physicochemical properties of amino acids; that is, glutamine is hydrophilic and would normally be found surface exposed. This is probably true in the case of Q31, as it is immediately adjacent to the nuclear localization signal.[64] Q31 replaced by leucine, a hydrophobic amino acid, would be disfavored at this position.
L100P[8]	*mo, rn*; L100P is not conserved; in humans equivalent amino acid S100; loop	Experimental: Reduces interaction with PDE4B and GSK3 (see main text for more details of effects of L100P). Binding region: APP, eIF3 p40, Kal-7, KIF5A, MAP1A, PCM1, TRIO.	Adjacent to the peptide region 101−135 shown to bind PDE4B. However, outside known GSK3β binding regions. As mentioned above, these have been incompletely mapped. However, a change from a Leu to a Pro could have structural consequences, since Pro is a conformationally important amino acid.

[a] All known DISC1 sequence variants are tabulated. The sequence conservation information was inferred on the basis of the ortholog multiple sequence alignment shown in Chubb et al.[3] *hs, Homo sapiens*; *pt, Pan troglodytes*; *ma, Macaca mulatta*; *bt, Bos taurus*; *cf, Canis familiaris*; *rn, Rattus norvegicus*; *mo, Mus musculus*; *tr, Takifugu rubripes*; *dr, Danio rerio*. Predicted location on secondary structure was based upon annotations made using methods described in Figure 1. Minor allele frequency for human common variants obtained from dbSNP[93] (1000 Genome phase 1 genotype data, release 08/04/2010). Binding region information obtained from the literature (refer to Table 1 and Figure 2).

cohort-specific nonsynonymous variants found in cases only were G14A, R37W, S90L, R418H, and T603I.

In a similar study, but focused on bipolar spectrum disorder, Song et al.[89] analyzed the regions of likely functional significance in *DISC1* in 504 patients with bipolar spectrum disorder (including a subset of cases with puerperal psychosis) and 576 ethnically similar controls. They found that five patients were heterozygous for ultrarare protein structural variants not found in the 576 controls ($p = 0.02$, one-sided Fisher's exact test) and shown to be ultrarare by their absence in a pool of 10 000 control alleles. In their sample, ultrarare (private) protein structural variants in DISC1 were associated with an estimated attributed risk of about 0.5% for bipolar spectrum disorder. However, they noted that the common variants were not associated with an elevated risk of bipolar disorder. The four identified ultrarare nonsynonymous variants found in cases only were S209R, R338Q, R418H, and T754S. Interestingly, the R418H variant was previously observed in their case-control study of *DISC1* in schizophrenia.

Following on from a previous linkage study on schizoaffective spectrum cases[107] and from an unpublished report that sequenced *DISC1* using 14 unrelated schizoaffective samples from the linkage study, two nonsynonymous coding SNPs in exon 11 were identified in two separate individuals. Green et al.[92] followed this up by sequencing exon 11 in 506 cases and in 1211 controls for variants that occurred only once. As a result, four

Table 3. DISC1 Subcellular Localization[a]

cellular location	details and associated functions of DISC1	refs
Centrosome	Involved in recruitment of kendrin, dynein, and dynactin subunits, LIS1, NDEL1, PCM1, ninein, and CAMDI to the centrosome. Also interacts here with PDE4B, PDE4D, NDE1, and BBS4. These interactions include those important in microtubule aster formation, neurite outgrowth, and neuronal migration.	27, 50, 65, 66, 74, 75, 79, 110, and 111
Cilia	Found at the base of primary cilia and appears to regulate their formation and/or maintenance. Knockdown of endogenous DISC1 leads to marked reduction of primary cilia. Other DISC1 interactors seen at the cilia include the BBS family and PCM1.	112
Cytoskeleton	Seen along both actin filaments and microtubules. Expression of truncated DISC1 leads to a disorganized microtubule network.	27, 50, and 60
Growth cones	Found here in the hippocampus and is involved in recruiting proteins including LIS1, NDEL1, 14-3-3ε, and girdin via kinesin-based transport along the cytoskeleton. Also important for axonal elongation.	60, 71, 72, and 76
Membranes	Found in membrane fractions where it interacts with APP, which is important in cortical precursor formation.	46, 63, and 80
Mitochondria	Found at mitochondria present on microtubules and regulates their trafficking. Can cause mitochondria to form "ring" structures. Interacts with mitofilin within the mitochondria and is required for correct electron transport chain, monoamine oxidase, and Ca^{2+} activity.	46, 59, 77, 80, 97, and 113
Nucleus	Found at the chromatin, promyelocytic leukemia bodies, and outer layers of nuclear membrane. Represses ATF4 transcriptional activity and transcription of N-cadherin and alters sleep homeostasis in *Drosophila*.	59, 64, 114, and 115
Synapse	Seen at the postsynaptic density along with PDE4 and NDE1. Interacts with and inhibits TNIK here, leading to degradation of key synaptic proteins. Also affects dendritic spine formation through modulation of PSD-95/Kal-7/Rac1 complexes here.	8, 73, 81, 110, and 114

[a] The regions where DISC1 has been shown to localize are provided, along with summary details of known roles or associated functions therein.

additional rare variants including two missense variants, T754S and P758R, were found only in cases. Note that the T754S variant was identified by Song et al.[89] in the same individual.

Since the discovery of these ultrarare mutations is fairly recent, there have not been any published studies thus far examining their functional effects. However, preliminary results indicate that R37W enhances DISC1 localization to the mitochondria and induces mitochondrial morphological abnormalities.[88] By taking into account the location and overlap of these mutations with known binding regions and along with predicted or known localization motifs, a few clues emerge for future experimental follow-up (Table 2).

3.3. Mouse DISC1 Missense Mutations. *N*-Ethyl-*N*-nitrosourea (ENU) is a powerful mutagen that induces single base DNA changes which can give rise to single amino acid substitutions. Applied to male mice, it provides a method for inducing and then screening for mutations in selected genes and rescuing these by breeding. Clapcote et al. did so for mouse *DISC1* exon 2 and identified and characterized two missense mutations that led to amino acid substitutions: Q31L and L100P that were associated with depressive and schizophrenic-like behavior, respectively.[8] Mice expressing either Q31L or L100P display inhibited cortical neuronal proliferation, aberrant neuronal migration, reduced dendritic spine density,[108] and lower total brain volume.[8] Mechanistically, both mutations reduce the interaction of DISC1 with its key interacting proteins PDE4B and GSK3.[8−10] Furthermore, the Q31L mutant inhibits PDE4 activity,[8] and L100P affects dopamine function.[109] Finally, while there is no evidence of altered enzymatic activity for L100P, the Q31L mutation results in increased GSK3 enzymatic activity.[9,10] Neither Q31L nor L100P is conserved between human and mouse, nor do they correspond to known clinical mutations (Table 2), but as noted their potential for structural disruption and proximity to functional domains and protein binding regions (Figure 2 and Tables 1 and 2) does allow testable inferences to be made as to their likely functional consequences.

4. DISC1 SUBCELLULAR LOCALIZATION

DISC1 has been shown to locate to multiple subcellular compartments including the centrosome, at the base of cilia, cytoskeleton, growth cones, membranes, mitochondria, nucleus, and the synapse (Table 3 and references therein). This multicompartmentalization of DISC1 within the cell implies that it performs different roles and may bind different proteins in the different cellular components and thus have roles in a number of cellular processes. DISC1 dysfunction, therefore, is likely to have multiple consequences for cellular function, neural development, and ultimately mental illness.

The dynamic mechanisms by which DISC1 locates to its various cellular components remains to be elucidated in many cases. In general, transport or localization of a protein to its subcellular compartment is facilitated either by binding to a dedicated receptor via a signal sequence, following which the receptor shuttles the molecular "cargo" into or out of a specific subcellular compartment, or indirectly via binding to a partner protein. For example, DISC1 and several of its interaction partners are transported to the growth cones via kinesin trafficking along the microtubules,[72,76] and Miyoshi et al.[74] proposed that kendrin localized DISC1 to the centrosome. Localization of DISC1 to the ciliary base, cytoskeleton, growth cones, membranes, mitochondria, and synapse may be mediated by partner proteins (Table 3). However, we cannot rule out that there exist localization motifs within DISC1 that are not yet defined or immediately clear-cut. By contrast, there are well-defined protein sequence motifs that confer nuclear localization and export for DISC1 that have been subject to testing, which we review below.

Two studies have to date formed the bulk of the work assessing nuclear localization and nuclear export signals in DISC1.[49,64] Millar et al.[113] initially found that the N-terminus of DISC1 comprising residues 1−358 was important for localization of DISC1 to the nucleus. Indeed, two putative NLS sequence motifs were previously noticed within the N-terminal

region:[17,18] the classical tetra-arginine NLS motif[116,117] at position 35-RRRR-38; and a bipartite motif,[117,118] that consists of two basic residues, a ten residue spacer, and another basic region consisting of at least three basic residues out of the next five, at position 331-RKWEPVLRDCLLRNRRQ-347. Utilizing this information, Sawamura et al.[64] confirmed that DISC1 lacking the N-terminal amino acid residues 1−45 did not locate to the nucleus, while the region containing the sequence 35-RRRRLARRP-43 was sufficient to send fused-GFP to the nucleus. Indeed, alanine substitution mutagenesis of the tetra-arginine motif 35−38 caused the mutated DISC1 to locate outside the nucleus with enrichment at the perinuclear regions.[64] They further confirmed that the bipartite NLS did not localize GFP to the nucleus. A previous study by Brandon et al.,[46] who performed loss-of-charge substitution mutagenesis on some of the arginine residues in the bipartite site, also did not see any significant effect on localization, although intriguingly nor did they report any effect upon deletion of the four consecutive arginine residues in the classical NLS motif when using a GFP-fused DISC1 construct. Possible reasons for this discrepancy could be due to methodological differences in the use or attachment of the GFP tag and/or technical issues of immunofluorescence staining (e.g., fixation, permeabilization, and/or use of primary antibody). However, a replication mutagenesis study would be useful to reconcile these observational differences.

Brandon et al.[46] also examined the role of two potential nuclear export signal (NES) sites in DISC1. Typically, these NES motifs are characterized by conserved leucine-rich sequence motifs.[86] However, there was no effect on DISC1 localization upon mutating the relevant leucine residues to alanines in both sites, regions 502-CDLTPLVGQLSLGQ-515 and 619-EGLEGLLSKLLVLSS-633.[46] While the former site between residues 502 and 515 was a likely candidate site because of the semiconservation of the leucine-rich sequence, its surrounding context, and location within a predicted α-helix, the latter site (amino acid residues 619−633) corresponds to a predicted coiled-coil region. In that case, the positions "a" and "d" that are occupied by most of the leucine residues in this motif would be buried. Sawamura et al.[64] showed that introduction of proline substitution mutations in place of the leucine residues within the leucine zipper motif spanning 607−628 altered the distribution of DISC1 from the nucleus to the cytoplasm. They additionally noted another potential NES motif 546-LQERIKSLNL-555 (apart from the two examined by Brandon et al.[46]) and showed that a serine substitution mutation at position 553 in place of leucine leads to distribution of DISC1(1−597) from a cytoplasmic to a nuclear localization, while this same motif tagged to GFP alone led to it being localized entirely outside the nucleus. The authors suggest that this was a functional effect of the NES. However, caution should be advised on this interpretation because, as for the site examined in Brandon et al.,[46] this motif is located within a PCOILS predicted coiled-coil helix, and consequently the leucine residues could be buried and inaccessible to exportin. Hence, it is possible that the observed functional effect was caused indirectly by structural disruption. A scan for additional potential nuclear export signals using the NetNES version 1.1[86] reveals three more potential NES sites at residue positions 385, 388, and 707. While positions 385 and 388 are also located within a coiled-coil helix and potentially buried, the site at position 707 appears a likely candidate NES site. Specifically, the study by la Cour et al.[86] noted that, apart from being leucine-rich, NES motifs in general reside in α-helices and predominate in regions that are rich in negatively charged residues and serines. All these characteristics are pertinent to the 707 site (686-LLGKVWEADLEACRLLIQSLQLQE-709). Interestingly, the common amino acid substitution polymorphism, S704C, is a context residue; that is, the role of leucine residues that are involved in directly binding exportin are further facilitated by other amino acids in their vicinity that influence molecular recognition, within the predicted NES.

5. DISC1 IN DOPAMINE AND GLUTAMATE SIGNALING

All antipsychotic drugs currently licensed for clinical treatment are united by their activity at dopamine receptor D2 (DRD2), with their ability to antagonize the receptor correlating with neuroleptic activity,[119] although higher receptor occupancies are known to correlate with unwanted side effects.[120] Results such as these gave rise to the highly prominent dopamine theory of schizophrenia[121] which postulates that the positive symptoms of schizophrenia arise from hyperstimulation of DRD2, while negative and cognitive symptoms arise from hypostimulation of DRD1 in the prefrontal cortex (PFC) (reviewed in ref 122).

More recently, genetic analysis has hinted that dopamine-related genes such as *catechol-O-methyl transferase* (*COMT*) may be associated with risk of schizophrenia.[123] Thus the reporting of epistatic interactions between the *DISC1* gene and *COMT*[124] provided the first evidence that DISC1 may participate in the therapeutically relevant dopamine pathway. Since then, in vivo analysis of this putative intersection of pathways has been performed. Notably, in utero suppression of DISC1 expression by short hairpin RNA (shRNA) in mice led to a reduction of extracellular dopamine in the PFC and apparent defects in the maturation of axon terminals of dopaminergic projections.[125] Furthermore, in a cell model, DISC1 knockdown reduced the numbers of primary cilia expressing dopamine receptors.[112]

It is well established that the dopamine enhancing agents amphetamine and methamphetamine can induce psychotic episodes similar to those in a psychotic episode of schizophrenia,[122] making it interesting to note that mice with suppressed levels of *DISC1* expression are more sensitive to methamphetamines in an activity assay.[125] The effect of amphetamine is also enhanced in other *DISC1* mouse models. Overexpressing amino acids 1−597, corresponding to the hypothetical protein truncation in the original Scottish family,[1,2] has dominant negative effects in cell models,[50,80] and likewise in transgenic mice, including an enhanced effect on locomotor activity relative to control mice.[126] This may arise in part because the mutant mice display significantly reduced dopamine, and a metabolite thereof, in the frontal cortex.[126] The ENU-induced amino acid substitution L100P[8] was also associated with a heightened response to amphetamines on hyperactivity: this could be suppressed by the typical antipsychotic haloperidol.[109] Mice expressing the DISC1(1−597) truncate also showed inhibited sensitization to methamphetamine when administrated in an escalating dose, designed to represent the pattern of use of methamphetamine abusers,[127] further emphasizing the potential pharmacological relevance of DISC1.

The other prominent theory of schizophrenia concerns hypofunction of glutamate signaling, derived in part from evidence that *N*-methyl-D-aspartate (NMDA) receptor antagonists lead to symptoms reminiscent of schizophrenia.[128] Based on this, clinical trials of metabotropic glutamate receptor type 2/3 (mGluR2/3) agonists for schizophrenia have begun with promising early

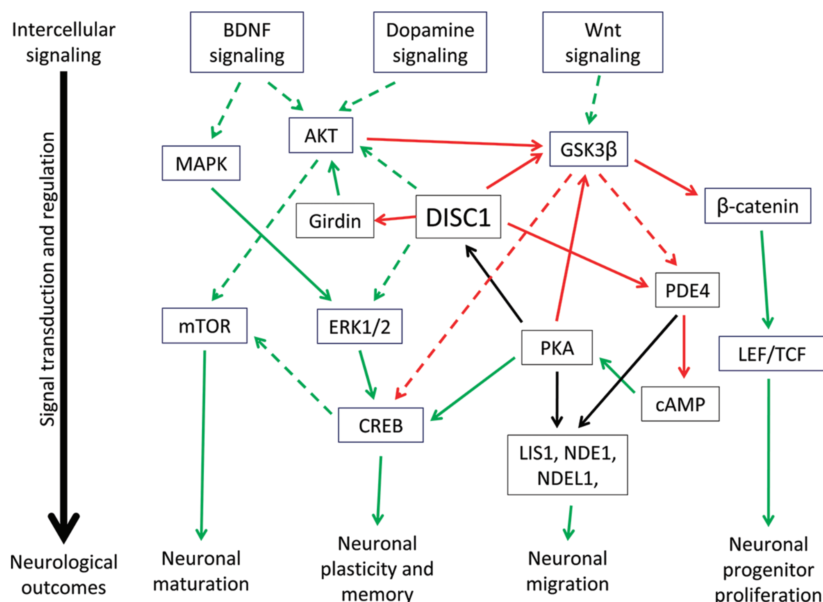

Figure 4. DISC1 therapeutic pathways. Neurologically relevant cellular signaling pathways influenced by DISC1 are shown. Green arrows depict activation enzymes, or otherwise enhancement of the target functions (for example, by leading to upregulated transcription of the protein). Red arrows depict inhibition or otherwise downregulation. Black arrows depict effects which do not fall easily into one of the above categories or that are not yet fully understood. Data on the role of DISC1 in these pathways was taken from refs 9−11, 36, 37, 70, 71, 98, 131, 158, and 200. Dashed arrows indicate indirect effects. Refer Abbreviations for full names and text for further details.

results.[129] The glutamate hypothesis is backed up by genetic evidence implicating proteins involved in glutamatergic function, including D-amino acid oxidase (DAAO), G72, and neuregulin 1 (NRG1) with schizophrenia (reviewed in ref 130). DISC1 was initially linked to glutamate signaling when knockdown of DISC1 in adult-born neurons of mice was seen to lead to an increase in spontaneous glutamatergic synaptic inputs.[131] Of the glutamate receptors, DISC1 appears to be involved in the regulation of GluR1 function, with prolonged DISC1 knockdown leading to a decrease in the size of dendritic spines expressing this receptor.[73] DISC1, through its interaction with TNIK (TRAF2 and NCK interacting protein kinase), also appears to modulate the degradation of GluR1 by the lysosome as well as its surface expression, with a detectable effect of inhibition of the DISC1-TNIK interaction on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor currents.[81]

Expression of DISC1 is also regulated by glutamate signaling, with use of NMDA receptor agonists leading to a decrease in expression of both DISC1 mRNA and synaptic DISC1 protein,[132,133] and knockdown of the NMDA receptor NR1 having a similar effect.[132] A direct effect of NMDA signaling on DISC1 appears to be the modulation of the synaptic complex of DISC1 with Kal-7 (kalirin-7) and PSD-95 (post-synaptic density protein 95)[73] which is involved in the regulation of dendritic spine structure. Further evidence of interaction between DISC1 and glutamate signaling comes from the ability of DISC1 overexpression to partially rescue overextended migration of adult born neurons caused by NMDA receptor antagonists in mice[133] and the fact that such an antagonist had a greater effect on the locomotor activity of mice expressing DISC1(1−597) both pre- and postnatally than on mice expressing it for shorter time periods.[126]

DISC1 function thus intersects in a complex manner with both of these therapeutically relevant signaling pathways (Figure 4), being modulated through NMDA receptor signaling and in turn affecting the surface expression of both dopaminergic and glutamatergic receptors. Excitingly, DISC1 and its complex may therefore provide an opening for therapeutic modulation of either or both dopaminergic and glutamatergic receptor function and signaling.

6. DISC1, GSK3, AND PDE4: A FUNCTIONAL COMPLEX WITH POTENTIAL THERAPEUTIC OUTCOMES?

The interest in glycogen synthase kinase 3 (GSK3) as a DISC1 interactor was sparked by functional studies performed by Mao et al.[11] GSK3 is an enzyme strongly implicated in psychiatric illness by virtue of its inhibition by lithium chloride, a common mood stabilizer and primary treatment for bipolar disorder.[134] Most attention thus far has focused on GSK3*β*, the ubiquitous isoform with the more severe mouse knockout phenotype,[135] but two other isoenzymes also exist, both of which have been implicated in neuronal outgrowth. GSK3α is coded for by a separate gene, and GSK3*β*2 is a neuronal specific alternative splice form of GSK3*β*.[136] Unlike the beta knockouts, GSK3α homozygotes are grossly normal, although brain specific deletion does result in decreased aggression, locomotion, and fear conditioning deficits.[135,137] This clear difference between the gross phenotype of the mice suggests that these similar proteins perform nonredundant roles in development, while there may be some overlap between interacting substrates.

GSK3*β* is a virtually ubiquitous enzyme present throughout hippocampal neurons, in dendritic spines, and present in both the cytosolic and synaptosomal subcellular fractions. The enzyme has a large array of substrates and multiple well demonstrated roles which include regulation of cell proliferation[138] and synaptic plasticity[139] with a peak in expression between embryonic day 18 and postnatal day 10, which corresponds to the major period of synapse formation. As DISC1 expression peaks at E14-E15 in mice,[140] a period which coincides with neurogenesis, a putative role for DISC1 interacting with GSK3 in cell

proliferation was investigated. DISC1 knockdown decreases and overexpression increases proliferation of hippocampal progenitor cells both in vitro and in vivo. Furthermore, the inhibitory effect of DISC1 knockdown can be reversed by pharmacological inhibition of GSK3. These effects are mediated via β-catenin stabilization and the Wnt signaling pathway, one of the major targets of GSK3β.[11] Indeed, DISC1 knockdown results in an increase in the activating autophosphorylation of GSK3β, while overexpression has the opposite effect. GSK3β binds directly to fragments 1−120 and 356−595 of DISC1 (Figure 2), and these DISC1 fragments inhibit GSK3β autophosphorylation and downstream substrate phosphorylation. A smaller peptide encompassing the highly conserved DISC1 residues 195−238 inhibits GSK3β autophosphorylation more potently than the commercial GSK3β peptide inhibitor L803-mts.[11] Recent data has suggested that the interaction between the two proteins may be dependent on DISC1 phosphorylation at mouse S710/human S713, which may lead to a developmental switch from proliferation to differentiation of neuronal precursors.[14] Thus, not only does DISC1 appear to bind GSK3β, but it also has functional effects on the activity level of the enzyme, suggesting implications for downstream signaling of GSK3β mediated pathways.

Phosphodiesterase-4 (PDE4) enzymes are the sole means of inactivating cAMP within the cell,[141,142] allowing fine spatial and temporal control of cAMP fluxes.[143] The four-lettered subtypes A−D are coded for by separate genes, each of which has multiple length splice isoforms (long, short, supershort) whose activity can be differentially controlled.[144−146] In addition, the interaction of DISC1 with the various isoforms of PDE4 is isoform-dependent. Members from all four-lettered subtypes of PDE4 immunoprecipitate with 100 kDa DISC1, with PDE4C2 and PDE4D3 (but not PDE4A5 and PDE4B1) being released under conditions of increased cAMP.[37] PDE4B did however have a similar dynamic interaction, when bound to 71 kDa DISC1[36] or a DISC1 construct lacking its N-terminal 187 amino acids.[37] PDE4 inhibitors are known to show antidepressant and antipsychotic-like like effects in rodent models,[147−149] and also affect dopaminergic signaling pathways in the striatum and frontal cortex by indirectly modifying phosphorylation of synthesis related pathways, key receptors, and downstream signaling molecules;[150,151] however, as has been previously observed,[6] side effects from nonisoform-selective PDE4-inhibitors prohibit their clinical use. Nevertheless, recent progress has been made in the identification of compounds with a high specificity for PDE4B,[152−154] PDE4D,[155,156] or specific long PDE4D subtypes.[155] Crucially, allosteric PDE4D-specific inhibitors had cognitive efficacy, but with reduced emetic side effects in rodents.[156,157] Together, these open the possibility that selective PDE4-inhibitors may possess therapeutic potential.

More recent work has suggested that PDE4 activity is also intrinsically linked to the outcome of GSK3-DISC1 interactions. Low doses of either rolipram, to inhibit PDE4, or of Thiadiazolidine (TDZD-8) to inhibit GSK3, have no effect on the pre-pulse inhibition (PPI) deficit or the hyperlocomotion that characterizes the L100P “schizophrenic-like” mutant mouse,[9] but combined acute treatment of DISC1 L100P with rolipram and TDZD-8 rescues both the PPI deficiencies and hyperlocomotion phenotypes.[10] In the Q31L “depressive-like” mice, which have a reduction in PDE4B activity, TDZD-8 alone improved PPI deficits, decreased immobile time in the forced swim test, and increased social motivation in a dose dependent manner. Both mutations of DISC1 lead to decreased coimmunoprecipitation of GSK3α and β isoenzymes, suggesting a reduced interaction of DISC1-GSK3 may be responsible for the phenotypes of these animals.[10] There is as yet no evidence to support selectivity of DISC1 binding for either isoform of GSK3. However, genetic inactivation of GSK3α results in a rescue of the behavioral phenotypes observed in L100P mice, and partially rescues the histological phenotype.[137] Lower inhibitory phosphorylation of GSK3α was also observed in the striatum of L100P animals, while there was no change in GSK3β phosphorylation or hippocampal forms of either isoenzyme.[9] These experiments suggest that deficits in the fine control of the interaction between DISC1, GSK3, and PDE4 may lead to susceptibility to psychiatric disorder, and the specific localization of the deficit may define the phenotype of the disorder. To add to this evidence, in neuronal-type cell culture experiments, both DISC1 overexpression and GSK3 inhibition curb the increase in PDE4 activity in response to a forskolin-induced increase in cAMP levels. GSK3 inhibition also tonically decreases PDE4 activity, leading to an increase in cAMP in treated SH-SY5Y cells.[158] The mechanism by which these changes occur are not yet clear, though phosphorylation of PDE4D by GSK3β has recently been demonstrated.[159] It is important to note that despite mounting evidence that these pathways interact functionally, there is as yet no direct evidence that DISC1, PDE4, and GSK3 all interact simultaneously within the same complex.

cAMP signaling is a further player in the outcome of interaction between DISC1, GSK3, and PDE4 (Figure 4). Protein kinase A (PKA) is an activator of PDE4 long isoforms[144] and also a substrate of GSK3β.[160] Furthermore, there is also evidence that cAMP signaling acts upstream of GSK3β.[161] Forskolin increases inhibitory phosphorylation of GSK3β, and decreases activity by 50% in primary cerebellar granule cells. These effects are blocked by administration of the PKA inhibitor H89 to cultures but are independent of ERK and AKT signaling. Indeed, purified PKA directly phosphorylates purified GSK3β in vitro, although immunoprecipitation experiments in vivo have not yet demonstrated a direct interaction.[162]

The high complexity, potential for redundancy, and ubiquitous nature of the signaling pathways involved in DISC1-PDE4-GSK3 signaling makes targeting of therapeutic compounds for these disorders difficult. Despite this, there are compounds available for use in both humans and animal models that show some therapeutic promise. Lithium remains a frontline treatment for bipolar disorder. There have been many behavioral studies performed on wild type rodents to assess the effects of lithium treatment, which suggest antidepressant, anxiolytic, and antimanic properties.[134,163] It is still unclear whether there is a definitive molecular mechanism by which lithium exerts its effects, but pharmacological and genetic manipulation has strengthened the case that inhibition of GSK3β is a rational therapeutic target. GSK3β heterozygous knockout mice recapitulate the behavioral effects of lithium-treated wild types, demonstrating decreased immobile time in the forced swim test, decreased exploratory behavior, and decreased amphetamine induced hyperlocomotion.[134,164,165] Lithium treatment of GSK3β heterozygous knockout mice results in impeded locomotion, while GSK3β homozygous knockouts are embryonic lethal,[164] suggesting that there is an optimal level of GSK3β inhibition for beneficial neuroactive effects. One of the key effectors of lithium treatment may be related to its effect on synaptic plasticity. Induction of long-term depression (LTD) results in an increase in GSK3β activity in CA1 of the hippocampus. GSK3β inhibitors prevented the induction of LTD in in vivo recordings

from 2 week old rat hippocampi.[166] Conversely, long-term potentiation (LTP) inhibits GSK3β activity in vivo and in vitro through inhibitory phosphorylation,[166,167] while transgenic mice overexpressing GSK3β have significant deficits in LTP and spatial learning deficits that are rescued by lithium treatment.[167]

Pharmacological inhibition of GSK3 by specific inhibitors produces very similar effects in mice to those exhibited in response to lithium treatment. Thiadiazolidine has anxiolytic and antimanic properties.[134,168] In mice with the dopamine active transporter knocked out, lithium antagonizes dopamine dependent hyperactivity, as do the specific GSK3 inhibitors SB216763, sodium valproate, and TDZD-8.[165] GSK3 specific inhibition in wild type mice also leads to decreased immobile time in the forced swim test,[137,168] one of the best replicated effects of lithium treatment.[163]

In cell culture and post-mortem studies, treatment with antipsychotic drugs also has effects on GSK3β activation. Short-term treatment of SH-SY5Y cells with clozapine results in increased inhibitory phosphorylation of GSK3β and an accumulation of β-catenin in the nucleus.[169] Acute risperidone, olanzapine, and clozapine treatment increases inhibitory GSK3β phosphorylation in multiple brain regions of relevance to psychiatric disorders. Interestingly, lower doses of risperidone have a greater effect on GSK3β phosphorylation than high doses. In the same paradigm, haloperidol has no effect.[170] The evidence for GSK3β playing an important role in multiple psychiatric disorders is multileveled and growing rapidly and is strengthened by its association with DISC1 and PDE4.

6.1. DISC1-GSK3-PDE4 and Neurotrophin Signaling. Signaling cascades crucial for the regulation of synaptic plasticity, formation of dendritic spines, neuronal complexity, differentiation, and survival by activation in response to neurotrophins[171−175] also interact with the DISC1-GSK3-PDE4 complex at multiple levels. Brain-derived neurotrophic factor (BDNF) is a molecule of particular interest in depression[176,177] and when administered centrally has antidepressant effects in multiple behavioral tests.[178] Chronic administration of antidepressants increases BDNF mRNA in the hippocampus.[176] Treatment of SH-SY5Y cells with BDNF leads to a decrease in GSK3β activity via AKT mediated inhibitory phosphorylation and results in increased phosphorylation of the cAMP response element binding protein (CREB), as does treatment of cells with lithium.[179,180] Overexpression of GSK3β blocks BDNF induced phosphorylation of CREB, except in the presence of lithium, which also causes a small increase in ERK1/2 and CREB phosphorylation in control (non-BDNF treated) cells.[173]

CREB is one of the major downstream effectors of neurotrophins and has similar antidepressant effects to BDNF when expressed in the hippocampus.[181] CREB is activated by chronic antidepressant treatment,[182] ERK (via ribosomal S6 kinase), and PKA by phosphorylation[173,183,184] and negatively regulated by GSK3β.[180] ERK and AKT signaling is activated by phosphorylation after chronic treatment with valproate, and short-term treatment with carbamazepine enhances basal and BDNF stimulated ERK1/2 and CREB phosphorylation.[171,173] Both of these drugs are commonly used mood stabilizers. Phosphorylation of AKT in response to valproate treatment is accompanied by an increase in inhibitory phosphorylation of GSK3β, while lithium treatment has this same effect without an increase in AKT phosphorylation.[185] This information leads to a central pathway inextricably linked to psychiatric disorder, by its outcomes (control of neuronal plasticity and survival), by its modulation at various points by antidepressant and mood stabilizing drugs, and by post-mortem evidence from human disease. Schizophrenic patients have 68% decreased levels of AKT protein in peripheral tissues and decreased levels in post-mortem frontal cortex and hippocampus when compared to a control population. There are concomitant decreases in inhibitory phosphorylation of GSK3β in the periphery and brain post-mortem tissue.[186]

To summarize, growth factors, particularly BDNF, bind to tyrosine kinase receptors and activate phosphoinositide-3-kinase (PI3K) and mitogen activated protein kinase (MAPK) cascades, resulting in phosphorylation of ERK1/2 and AKT (Figure 4). Activation of ERK leads to activating phosphorylation of CREB, which binds to CRE response elements in multiple genes, resulting in transcription of neuronal plasticity and survival related genes. Concomitant activation of AKT results in inhibition of GSK3β, allowing CREB activation. This basic pathway can be stimulated by mood stabilizing and antidepressant drugs, by either direct inhibition of GSK3β or upstream activation of ERK and AKT.[183] This pathway therefore has three major outcomes with implications for psychiatric disease: activity changes downstream of ERK such as CREB mediated gene transcription, activity changes downstream of AKT, including activation of mammalian target of rapamycin (mTOR) which phosphorylates eukaryotic translation initiation factor 4E (eIF-4E) binding protein to regulate translation,[163,187] and changes in DISC1 associated GSK3 and PDE4 activity. As an example of the latter, GSK3β inhibits the accumulation of β-catenin through phosphorylation leading to ubiquitination,[134] while DISC1 has been proven to stabilize β-catenin through an inhibitory effect on GSK3β.[11] β-catenin is implicated in Wnt independent dendritic morphogenesis[188] and synaptic plasticity,[189] and this may go some way to explaining the changes in synaptic complexity seen in DISC1 mutant mice.

6.2. DISC1: Effects on AKT Signaling Independent of GSK3. DISC1 is capable of modulating these pathways by means likely independent of its direct association with GSK3. Small interfering RNA (siRNA) knockdown of DISC1 in primary cortical cultures results in significantly decreased phosphorylation of ERK1/2 (pERK) and AKT (pAKT).[98] Viral transduction of both the S704 and C704 variants of DISC1 results in an increase in pERK1/2 and pAKT. These effects are more significant when the Ser variant is overexpressed: the pAKT increase after overexpression of the Cys variant is nonsignificant.[98] In rescue experiments, overexpression of Ser DISC1 variant compensated for the decrease in pERK1/2 resulting from siRNA mediated DISC1 knockdown. There was a nonsignificant return to basal pERK1/2 levels when the Cys variant was overexpressed: a similar pattern was observed with pAKT.[98] Thus, it seems that DISC1 plays a similar biological role to that of mood stabilizers in this paradigm and is capable of enhancing ERK and AKT activation, with the potential to exert effects on synaptic plasticity and neuron survival as described earlier.

In addition, DISC1 forms a ternary complex with kinesin-1 and Grb2, an adapter molecule linking neurotrophin binding at tyrosine kinase receptors to downstream effectors such as Ras by recruiting guanine-nucleotide exchange factors.[72] Ras acts upstream of AKT and ERK in the MAPK and PI3K signaling pathways.[190] DISC1 and Grb2 are partially colocalized along microtubules in the central region of primary rat hippocampal axon growth cones: DISC1 knockdown prevents Grb2 from accumulating in this area. Growth cone localization of Grb2 is

rescued by overexpression of wild type DISC1, but not by overexpression of DISC1 with a mutated SH3 binding domain.[72] However, unlike the observations described above in unstimulated neurons, knockdown of DISC1 has no effect on ERK2 phosphorylation in neurotrophin-3 (NT-3) treated PC12 cells. Under NT-3 stimulated conditions, DISC1 knockdown results in an axon elongation defect which is not rescued by overexpression of the SH3 domain mutated DISC1.[72] Conversely, the axon elongation defect seen in non-NT-3 stimulated neurons is rescued by both wild type and mutated DISC1, suggesting that the DISC1/Grb2 interaction is only critical for NT-3 stimulated axon elongation.[72] These studies suggest a possible pathway by which DISC1 may alter ERK and AKT phosphorylation, but there are some discrepancies between the two pieces of work which have yet to be resolved. It may be that the differences between the effects of DISC1 observed in unstimulated neurons arise from methodological differences (such as differences in cortical versus hippocampal neurons), or the situation may be more complex than is presently appreciated.

Knockdown of DISC1 in the adult mouse brain results in a cellular phenotype very similar to that of phosphatase and tensin homologue (PTEN) suppressed mice.[191] Adult born neurons in the dentate gyrus microinjected with a *DISC1* shRNA-containing oncoretrovirus have larger cell bodies, an increased number of ectopic primary dendrites, more complex dendritic arborization, and an accelerated rate of synapse formation and may overmigrate through cortical layers.[131] In the PTEN mutant mice, cells with ectopic dendrites exhibit an increase in pAKT and phosphorylated GSK3β, whereas cells without ectopic dendrites have no pAKT changes.[191] This phenotypic similarity led to the hypothesis that DISC1 regulates the development of adult born neurons through activation of AKT signaling. Although there is no direct binding between DISC1 and AKT,[70] multiple lines of evidence suggest a functional interaction. DISC1 directly binds girdin, a protein which is expressed during differentiation of adult born neurons, and binds to AKT to potentiate AKT activation.[192] There are important points of difference between the experimental approaches taken and the conclusions drawn, including the domains of DISC1−girdin interaction and of the downstream effects on AKT signaling that remain to be resolved, but the potential implications for DISC1 modulation of AKT signaling via girdin are provocative[193] and merit follow up. Thus, DISC1 single cell knockdown resulted in an increased immunostaining of pAKT in immature post-mitotic neurons; overexpression of girdin was sufficient to elevate levels of pAKT and pS6 without insulin stimulation, but this was antagonized by coexpression of DISC1; while overexpression of DISC1 alone had no effect on pAKT levels in either insulin stimulated or unstimulated cells.[70] This is in contrast to earlier observations in cortical neurons, where DISC1 overexpression in unstimulated cells resulted in an increase in pAKT.[98] Girdin overexpressing neurons (thus increasing AKT signaling) exhibit a cellular morphology and dendritic arborization strikingly similar to PTEN and DISC1 knockdown neurons, as do neurons expressing a constitutively active form of AKT. Both these neuron types exhibit a migration and integration phenotype, but less severe than is observed with DISC1 knockdown.[70] Rapamycin can be used to inhibit mTOR, a kinase enzyme that functions downstream of AKT. mTOR regulates translation by phosphorylating eIF-4E. Rapamycin application fully rescued the effects of DISC1 knockdown or girdin overexpression in adult born neurons, while having no effect on S6 phosphorylation or morphology in control cells. Application of SB216763, a specific inhibitor of GSK3β, had no significant rescue effect, implicating mTOR as the pathway of interest with respect to differentiation, growth, and integration of adult born neurons.[70]

7. FUTURE DIRECTIONS AND CONCLUDING REMARKS

To conclude, in the first 10 years of research beyond the discovery of DISC1, much has been learned about the genetics and biology of this intriguing and novel scaffold protein. DISC1 clearly plays a fundamental role on neurodevelopment and neurosignaling, processes which are abrogated to greater or lesser extent by clinical and experimental mutations in both human subjects and laboratory animals. In some, but not all, examples, it is already possible to draw direct, if partial, conclusions about the pathogenic mechanisms of sequence variants and, through bioinformatics analyses, make useful experimental predictions. The multiple interacting protein partners of DISC1 identify pathways and potential targets of relevance to schizophrenia, bipolar disorder, and potentially more generally for cognition and depression. The recent evidence highlighting both cAMP and Wnt signaling, the potential impact on AKT signaling, and the accumulating evidence for a role in both dopaminergic and glutamatergic signaling put the DISC1 pathway firmly on the table as a promising target for rational drug development. But this is just the start of a much longer and challenging process. Without a fuller knowledge and understanding of the biophysical and structural properties of native and mutant DISC1, on its own and bound to physiologically relevant protein partners, there will be strict limits to what can be inferred or deduced with regards to the structure−function relationships necessary for a more complete understanding of DISC1 biology, and the scope for therapeutic intervention.

■ ASSOCIATED CONTENT

S Supporting Information. Additional figures showing MetaPrDOS prediction of disorder in DISC1 using a 5% false positive rate threshold and application of six coiled-coil prediction methods on DISC1; table presenting interaction regions mapped by yeast two-hybrid experiments only. This material is available free of charge via the Internet at http://pubs.acs.org.

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All authors conceived, designed and wrote the manuscript, with section-based major contributions as follows: D.C.S. Introduction and Sections 1-4, Figures 1 and 2; B.C.C. Sections 5 and 6; N.J.B. Introduction and Sections 1-5, Figures 2-4; D.J.P. Introduction and Sections 1, 2 and 7.

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ABBREVIATIONS

AKT, Rac-alpha serine/threonine-protein kinase; APP, amyloid precursor protein; ATF4/ATF5, activating transcription factor 4/5, ATF4 also known as CREB2; BBS1/BBS4/BBS8, Bardet—Biedl Syndrome 1/4/8; BDNF, brain-derived neurotrophic factor; CAMDI, coiled-coil protein associated with myosin II and DISC1; COMT, catechol-*O*-methyl transferase; CREB, cAMP response element binding protein; DAAO, D-amino acid oxidase; DBZ, DISC1-binding zinc-finger, also known as Su48 and ZNF365A; DISC1, disrupted in schizophrenia 1; Dixdc1, DIX domain containing-1; DRD1/DRD2, dopamine receptor D1/2; eIF3 p40, eukaryotic translation initiation factor 3, p40 subunit; eIF-4E, eukaryotic translation initiation factor 4E; ERK, extracellular signal-regulated kinase; FEZ1, fasciculation and elongation protein zeta-1; Girdin, girders of actin, also known as KIAA1212; Grb2, growth factor receptor-bound protein 2; GSK3α/GSK3β, glycogen synthase kinase 3-α/β; (m)GluR, (metabotropic) glutamate receptor; Kal-7, kalirin 7; kendrin, also known as pericentrin/B; KIF5A, kinesin family member 5A; LEF/TCF, lymphoid enhancer factor/T cell factor; LIS1, lissencephaly 1, also known as PAFAH1B1; MAP1A, microtubule-associated protein 1A; MAPK, mitogen activated protein kinase; MIP-T3, microtubule-interacting protein associated with TRAF3; mTOR, mammalian target of rapamycin; N-CoR, nuclear receptor corepressor; NDE1, nuclear distribution protein E homologue 1 or nuclear distribution element 1, also known as NudE; NDEL1, NDE-like 1, also known as nudel; NMDA, *N*-methyl-D-aspartate; NRG, neuregulin; PCM1, pericentriolar material 1; PDE4B/PDE4D, cAMP-specific 3′,5′-cyclic phosphodiesterase 4B/D; PI3K, phosphoinositide-3-kinase; PKA, protein kinase A; PSD-95, post-synaptic density protein 95; PTEN, phosphatase and tensin homologue; Rac1, Ras-related C3 botulinum toxin substrate 1; TNIK, TRAF2 and NCK-interacting protein kinase; TRIO, triple functional domain protein

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