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Rare disruptive variants in the DISC1 Interactome and Regulome: association with cognitive ability and schizophrenia

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Schizophrenia (SCZ), bipolar disorder (BD) and recurrent major depressive disorder (rMDD) are common psychiatric illnesses. All have been associated with lower cognitive ability, and show evidence of genetic overlap and substantial evidence of pleiotropy with cognitive function and neurotism. Disrupted in schizophrenia 1 (DISC1) protein directly interacts with a large set of proteins (DISC1 Interactome) that are involved in brain development and signaling. Modulation of DISC1 expression alters the expression of a circumscribed set of genes (DISC1 Regulome) that are also implicated in brain biology and disorder. Here we report targeted sequencing of 59 DISC1 Interactome genes and 154 Regulome genes in 654 psychiatric patients and 889 cognitively-phenotyped control subjects, on whom we previously reported evidence for trait association from complete sequencing of the DISC1 locus. Burden analyses of rare and singleton variants predicted to be damaging were performed for psychiatric disorders, cognitive variables and personality traits. The DISC1 Interactome and Regulome showed differential association across the phenotypes tested. After family-wise error correction across all traits (FWERacross), an increased burden of singleton disruptive variants in the Regulome was associated with SCZ (FWERacross, P = 0.0339). The burden of singleton disruptive variants in the DISC1 Interactome was associated with low cognitive ability at age 11 (FWERacross, P = 0.0043). These results identify altered regulation of schizophrenia candidate genes by DISC1 and its core Interactome as an alternate pathway for schizophrenia risk, consistent with the emerging effects of rare copy number variants associated with intellectual disability.

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INTRODUCTION
Schizophrenia (SCZ), bipolar disorder (BD) and recurrent major depressive disorder (rMDD) affect tens of millions of people worldwide. These disorders are moderately heritable and family history is a strong predictor of risk. Genome-wide association studies (GWAS), structural variant analyses and genome sequencing studies have identified that common single-nucleotide variants (SNVs), low penetrant rare SNVs, moderate to high penetrant copy number variants (CNVs) and potentially causal variants (SNVs), low penetrant rare SNVs, moderate to high penetrant copy number variants (CNVs) and potentially causal de novo mutations each play a role in the genetic etiology of SCZ and BD and, to a lesser extent, in rMDD.1–4

There is now strong evidence for shared genetic risk across traditional diagnostic boundaries supporting the observation of ‘mixed’ diagnoses families.5,6 GWAS studies capture common, ancient variation and point to an additive, polygenic architecture that transcends psychiatric diagnoses to predict cognitive ability variables.1,7,8 Lower cognitive function, both premorbid and post-onset, has been associated with these disorders, and recently polygenic risk score analysis has suggested a small, but significant, genetic correlation between risk for major mental illness and cognitive ability.9

In a complementary fashion to common variants identified in GWAS, rare variants have been identified that segregate with psychiatric disorder in a quasi-Mendelian manner and impact upon normal cognitive function.10,11 One such example is a balanced t(1;11) (q42;q14) translocation in the Disrupted in Schizophrenia 1 (DISC1) gene, which was identified in a large Scottish pedigree highly burdened with SCZ, BD and rMDD.12 Independent reports of linkage and association have since reported evidence for region-wide association of DISC1 variants, or more commonly haplotypes, with these and other psychiatric disorders as well as for cognitive and neuropsychological traits.13–15 Although DISC1 itself is not a GWAS significant finding, its interactor PDE4B and regulated gene NRXN1 are reported as significant.16,17 Convergent functional genomics approaches integrating the functional and genotypic data continue to support involvement of DISC1 disruption in schizophrenia and related biological pathways.18,19

Recently, we reported deep sequencing of the DISC1 locus (528 kb) in 1542 samples that identified 2010 rare variants, of which ~60% were novel.17 We identified a common intrinsic variant with region-wide association for rMDD, and a rare missense mutation (R37W), previously reported in a SCZ case,20

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in an individual with rMDD and in additional family members with mental disorders.\(^\text{17}\) Burden analysis also identified nominal associations with measures of depressed mood and cognitive ability at age 11, age 70 and cognitive ageing (change in cognitive ability at age 11 and change between age 11 and 70).\(^\text{17}\) Motivated by these findings, we hypothesized that further insights might emerge from a directly comparable study of the DISC1 pathway genes.

Molecular studies have shown that DISC1 functions as a scaffold protein that is critical in cell signalling, neuronal development and ontogenesis through multiple protein-protein interactions.\(^\text{23,26}\) DISC1-interacting partners (DISC1 Interactome) are enriched for proteins known to be involved in neural proliferation, migration, signaling and synaptic function.\(^\text{14,21,22,24,27}\) Positive case-control associations have been reported with psychiatric disorders for the following DISC1 Interactome genes: ATF4, CIT, NDE1, PCMK1, PDE4B, PDE4D and YWHAE.\(^\text{15}\) In addition, structural rearrangements of PDE4B and NDE1 have been reported in patients with SCZ.\(^\text{28,29}\)

Table 1. DISC1 (Number 1) Interactome and Regulome

| DISC1 Interactome | DISC1 Regulome |
|-------------------|----------------|
| AKAP9             | KCNQ1          |
| AP4B1             | ACTG2          |
| AP4M1             | DVL2           |
| APP               | DLGAP1         |
| ATF4              | ANKRD16        |
| ATF5              | APP2L2         |
| ATF7IP            | DUSP6          |
| CCDC88A           | ARC            |
| CDCL5             | DYNL1L         |
| CDK5RAP3          | EGR2           |
| CEP290            | EGR3           |
| CIT               | EGR4           |
| CLIC1             | MARCH3         |
| CLU               | MCPH1          |
| CTNB1             | EGRB2          |
| DCTN1             | MEG3           |
| DCTN2             | MODIC          |
| DISC1             | MPP7           |
| DISC1             | MPRR1B         |
| DIXDC1            | MPRR3K         |
| DPYS13            | MPRR5K         |
| DSY             | MPRR5P         |
| EIF3H             | MYRIP          |
| EXO4C             | NAPB           |
| FBXO1             | NAPI           |
| FEZ1              | NGRI1          |
| GNB1              | NGRI2          |
| GRB2              | NGRI3          |
| GRIPAP1           | NGRI5          |
| GSX3             | HSPH1          |
| TSN1              | HSPA2          |
| KALRN             | HSPA3          |

*Genes with a nominally significant burden p-values for schizophrenia (16 of 154 genes in the DISC1 Regulome). These gene level results did not survive family-wise error rate correction across all tests.\(^\text{16}\) Genes with a nominally significant burden p-values for cognitive ability at age 11 (4 of 59 genes in the DISC1 Interactome).
community-dwelling, generally healthy older people from the Lothian Birth Cohort of 1936 (LBC1936), as described previously.17 A total of 213 genes were selected for sequence analysis (Table 1, Supplementary Information: gene selection, Supplementary Table S1). The DISC1 locus (DISC1, TSNAK and TSNAK-DISC1) and 56 direct DISC1 protein–protein interactors defined the DISC1 Interactome gene set. A total of 154 additional genes related to DISC1 expression from previous microarray analyzes comprised the DISC1 Regulome gene set. Genomic regions comprising ~11.7 Mb (3.3 Mb exons) were captured using a custom solution capture probe set (Roche NimbleGen, Pleasanton, CA, USA). Each sample capture was sequenced using a HiSeq2000 sequencer (Illumina, San Diego, CA, USA). Sequence reads were aligned to the human NCBI Build 36 (hg18) reference using BWA.36 Variant calling was performed using GATK,37 and high-quality SNVs were filtered by standardized filtering parameters. Using PLINK,38 we applied data quality control filters as described previously to exclude samples and SNVs that introduce bias (Supplementary Figures S2–S5). Sanger sequencing was used to optimize the quality control filters and exclude all identified false positive SNVs from further analysis. SNVs were matched to hg19 coordinates using liftOver from UCSC, and ANNOVAR40 was used for variant annotation based on the human reference genome hg19 (RefSeq). Variant filtering was carried out by all algorithms (MAF1%) and singletons in these mutation classes was assessed in each of the case cohorts and a combined cohort of all diagnoses; and for cognitive measures: cognitive ability at ages 11 and 70, change in cognitive ability, crystallized cognitive ability and general cognitive ability; and personality traits: neuroticism, anxiety and depression (Supplementary Information: rare variant burden analysis).

RESULTS

Targeted sequencing and genetic discovery in 213 DISC1 Interactome and Regulome genes

A total of 1464 samples (95%) were sequenced to a minimum coverage depth of 20x across at least 80% of the targeted bases (Supplementary Table S2). Coverage was uniform across all sample groups (Supplementary Figure S1). Following sequence- and variant-based quality filters, 196 080 SNVs in 1446 samples (211 cases of SCZ, 169 cases of rMDD, 195 cases of BD, and 871 controls from the LBC1936) remained for further analyses (Supplementary Table S2 and Supplementary Figures S2–S5). Of the 196 080 SNVs, 78% have a MAF less than 1%. Only 40% are reported in the 1000 Genome Project European subset (Supplementary Table S3). On the basis of ReSeq functional annotations using ANNOVAR, 169 905 SNVs mapped to introns, 5410 to 3’ or 5’ UTRs, and 4523 to coding regions. Of the 4523 exonic variants, 1893 were functionally classified with respect to coding potential as silent variants, 2569 as missense, and 41 as nonsense. A further 24 SNVs were annotated as splice site variants. SNVs showing greater functional impacts on protein function are more likely to be rare: 100% of nonsense and 92% of splice site variants have MAF <1%, compared to 79% of silent and 78% of intronic variants.

Analysis of genetic variation in the DISC1 Interactome and Regulome with psychiatric illness

Rare functional variant analysis in the DISC1 Interactome. There was no significant burden of rare disruptive, NSstrict or NSbroad variants in SCZ, BD, or rMDD nor in a combined cohort of all diagnoses compared to controls in the DISC1 Interactome (Supplementary Table S6). There was a nominal association of
Fewer rare disruptive variants in SCZ (unadjusted \( P = 0.0188 \)), but no significant difference between the accumulation rate of rare variants for any diagnosis after Family-Wise Error Rate (FWER) correction (Supplementary Table S7). None of the proportions of \( N_{\text{strict}} \) and \( N_{\text{broad}} \) rare or singleton variants deviated from the null hypothesis after FWER across correction.

The gene-wide burden of non-synonymous coding changes was nominally, but not significantly increased in psychiatric disorders (unadjusted \( P = 0.0048 - 0.0488 \)) for several DISC1 interactome genes. None survived correction for multiple testing (Supplementary Table S8).

**Rare functional variant analysis in the DISC1 Regulome.** We analyzed the burden and accumulation rates of rare and singleton functional variants in the DISC1 Regulome. For SCZ compared to control samples, we observed a significantly increased burden of singleton disruptive variants (unadjusted \( P = 0.0019 \), FWER within \( P = 0.0069 \), FWER across \( P = 0.0339 \), OR = 1.3162, SE = 0.0941; Figure 1 and Supplementary Table S9), and a nominally higher accumulation rate (4.13-fold, unadjusted \( P = 9.00 \times 10^{-4} \), FWER within \( P = 0.0185 \), FWER across \( P = 0.0965 \), Supplementary Table S10). In addition, the accumulation rate of rare disruptive variants, as opposed to singleton disruptive variants, was 3.47-fold higher in SCZ cases than in healthy controls and remained significant after multiple test correction (unadjusted \( P = 1.68 \times 10^{-5} \), FWER within \( P = 1.00 \times 10^{-5} \), FWER across \( P = 0.0022 \), Supplementary Table S10).

Unlike singleton disruptive variants, although the burden of rare disruptive variants in SCZ was nominally significant, and survived FWER correction for all tests within the trait, it did not meet the threshold for tests across all traits (unadjusted \( P = 0.0061 \), FWER within \( P = 0.0228 \), FWER across \( P = 0.0863 \), Supplementary Table S9). We also observed a nominally higher proportion and burden of \( N_{\text{strict}} \) singleton and rare variants in SCZ and disruptive singleton and rare variants in combined cases compared to controls, but none survived FWER for all tests across all traits (Supplementary Tables S9 and S10). There was no evidence for an increased overall burden in rMDD, BD or combined cases compared to controls after FWER correction across all traits.

At the gene-wide level, *Translin-associated factor X interacting protein 1 (TSNAXIP1)* showed greater burden of \( N_{\text{strict}} \) singletons in rMDD (unadjusted \( P = 1.29 \times 10^{-4} \), FWER within \( P = 0.0253 \)) and \( N_{\text{strict}} \) rare variants in SCZ (unadjusted \( P = 2.22 \times 10^{-4} \), FWER within \( P = 0.0410 \), Supplementary Table S11) compared to controls. However, these results did not survive correction for all tests (rMDD FWER across \( P = 0.0864 \), SCZ FWER across \( P = 0.1600 \)). *TSNAXIP1* has 16 exons encoding 712 amino acids. We validated 17 rare coding variants in *TSNAXIP1* in all carriers, including 1 splice site, 1 nonsense and 15 missense variants (Figure 2 and Supplementary Table S12). Of these 17 rare substitutions, 4 were previously reported in the 1000 Genomes Project European subset. In total, 7 rare variants in *TSNAXIP1* including 2 disruptive and 5 predicted damaging missense variants contributed to the gene burden analysis of \( N_{\text{strict}} \) variants in rMDD and SCZ. In a ‘leave-one-out’ approach, we determined that the nonsense mutation at chr16:66405794 (rs146214814, p.R46X) located in exon 2, contributed most to the higher burden of \( N_{\text{strict}} \) variants in SCZ. Relative to controls, this disruptive variant had a 3.58-fold higher allele frequency in SCZ (0.0146 vs 0.0041) and was not observed in any other mental illness cohort. Further information on the neurobiology of *TSNAXIP1* is given in Supplementary Information: *TSNAXIP1*.

Burden analysis of coding variants on quantitative cognitive ability and personality traits associated with psychiatric illness. We found that a significantly higher burden of singleton disruptive variants in the DISC1 Interactome was associated with lower cognitive ability assessed by Moray House Test (MHT) scores at age 11 (unadjusted \( P = 9.35 \times 10^{-5} \), FWER within \( P = 0.0005 \), FWER across \( P = 0.0043 \), \( \beta = -7.1141 \), SE = 3.6863; Figure 3 and Supplementary Table S13). The burden of \( N_{\text{strict}} \) singletons in the Interactome gene set was associated with lower MHT scores at age 11 (unadjusted \( P = 0.0003 \), FWER within \( P = 0.0017 \), FWER across \( P = 0.0122 \), \( \beta = -2.7865 \), SE = 1.2877). In addition, although these did not survive FWER across correction, nominally significant associations in the burden of disruptive singletons were observed with MHT scores at age 70 (unadjusted \( P = 0.0056 \), \( \beta = -6.6785 \)), National Adult Reading Test (unadjusted \( P = 0.0051 \), \( \beta = -6.9970 \)) and General Fluid Intelligence (unadjusted \( P = 0.0293 \), \( \beta = -0.5152 \)). Interestingly, there were nominally significant associations between the burden of rare functional variants and increased symptoms of neuroticism (Disruptive singletons:...
unadjusted $P = 0.0154$, $\beta = 6.5671$), anxiety (NS strict rare variants: unadjusted $P = 0.0349$, $\beta = 0.1394$) and depression (NS strict singletons: unadjusted $P = 0.0431$, $\beta = 0.2587$). At the gene-wide level, no association was found between the variability in cognitive ability or personality scores and the burden of damaging or disruptive variants in any specific gene of the DISC1 Interactome after FWER across correction (Supplementary Table S14).

In the analysis of the DISC1 Regulome, we observed a burden of NS strict singletons associated with lower MHT scores at age 70 (unadjusted $P = 0.0014$, $\beta = -1.7895$; Figure 3 and Supplementary Table S15) that withstood FWER correction for all tests within the trait (FWERwithin $P = 0.0079$), but not all tests across all traits (FWERacross $P = 0.0609$). The burdens of NS strict and NS broad variants were nominally significantly associated with greater decrease in cognitive ability between the ages of 11 and 70 (NS strict singletons: unadjusted $P = 0.0131$, $\beta = -1.4338$; NS broad singletons: unadjusted $P = 0.0014$, $\beta = -0.3175$; NS broad rare variants: unadjusted $P = 0.0280$, $\beta = -0.0010$). At the gene-wide level, the strongest association with cognitive function was observed with rare and singleton NS strict variants in CACNA1C, but this did not pass FWER across correction (Supplementary Table S16).

**DISCUSSION**

Encouraging progress towards delineating the genetic architecture of psychiatric disorders has been made and roles for both common, rare and de novo mutations established. Rare variants of high impact can provide valuable mechanistic insight. Recent case-control deep sequencing studies indicate that in individuals with SCZ rare loss-of-function variants are enriched in genes related to synaptic function,31 in target genes of the FMRP32 and in genes known to be associated with SCZ.46 The biological impacts of several DISC1 missense variants identified through deep sequencing have been demonstrated.30,47 We previously reported the discovery of rare disruptive DISC1 variants in individuals with psychiatric illness and demonstrated the biological impact of the p.R37W variant.17 Here we report the association of both clinical diagnoses and cognitive ability with rare variants in the DISC1 Interactome and the DISC1 Regulome.

Before discussing these positive findings, we first consider some limitations of the study. Although the sample size was large by current standards, these numbers are modest in size for comprehensive rare variant detection.17,48 We were unable to perform sex-specific analyses in our study given our sample size. Similar analyses may be important in our understanding of the relationships between genetic variants and gene expression particularly in psychiatric illness, given reports of sex-specific differences in gene expression in the brain49,50 but also due to reports of sex-specific differences in association of variants and haplotypes in DISC1,51–53 the success of the CONVERGE strategy that relied on mapping loci for severe depression within a female-only cohort54 and the differences in disease presentation between sexes that have likewise been reported.55 Burden analysis increases the power of analyses in such small samples, but the
rules for annotating rare variants as ‘damaging’ are far from foolproof: biological validation is required. Last, but not least, whole genome sequencing of all 1543 individuals, while ideal, was beyond the scope of our resources. Targeted capture sequencing was a practical option, but it is likely that relevant variants will have been missed by virtue of poor capture. It is also almost certainly the case that our list of bona fide DISC1 interactors is incomplete, and that contra wise, not all members of the Regulome that met our inclusion criteria will be regulated by DISC1 in practice.

Acknowledging these limitations, there were findings of note. No association was seen between rare variants in the DISC1 Interactome and any psychiatric diagnosis. There was, however, a significant excess of singleton disruptive variants in the DISC1 Regulome associated with SCZ, but not with BD or rMDD. We have shown that disruptive and NS staffing singleton variants in the DISC1 Interactome show significant association with cognitive ability at age 11. These classes of variants are also nominally associated in the DISC1 Regulome with cognitive ability at age 70 and change in cognitive ability between age 11 and 70. The DISC1 Regulome gene set was assembled from genes that show both i) altered expression in response to genetic variation in DISC1 or its interactors, or are themselves protein interactors of the core complex, and ii) evidence of association with psychiatric illness from candidate gene studies, or some of the earliest genome-wide association studies. We note that in this study, we found nominal association of rare Regulome variants with both increased schizophrenia risk and lower adult cognitive ability, particularly in older age. This mirrors the observation of association with single disruptive Regulome variants and SCZ in our sample suggests a greater impact of glutamate dysregulation in this disorder than BD or rMDD. A role for DISC1 in glutamate-related processes has previously been suggested in both a mouse model and in the t(1;11) translocation family. Comparison of the GO terms associated with both the DISC1 Interactome and Regulome reveals largely independent GO term associations with a very limited set of intersecting terms focused on negative regulation of cellular process, protein binding, and cell projections (Supplementary Figures S9).

In conclusion, and despite the limitations, these findings provide further genetic evidence to support the impact of both DISC1-interacting proteins and genes whose expression is modulated by genetic variants in the DISC1 pathway on schizophrenia.

CONFLICT OF INTEREST

WRM has participated in Illumina sponsored meetings over the past 4 years, and received travel reimbursement and an honorarium for presenting at these events. Illumina had no role in decisions relating to the study/work to be published, the data collection and analysis of the data, and the decision to publish. WRM has participated in Pacific Biosciences sponsored meetings over the past 3 years and received travel reimbursement for presenting at these events. WRM is a founder and shareholder of Orion Genomics. WRM is a member of the scientific Advisory Board of RainDance, Inc. The remaining authors declare no conflicts of interest.

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AUTHOR CONTRIBUTIONS

DJP and WRM designed the study, are PIs on the grant funding, supervised the study and supported the analysis. ST, SM, PT, VM, MLC and MK carried out the collection and analysis of the data, and the decision to publish. WRM has participated in presenting at these events. WRM is a founder and shareholder of Orion Genomics. WRM is a member of the scientific Advisory Board of RainDance, Inc. The remaining authors declare no conflicts of interest.

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