Archival Report

Biological Psychiatry: Celebrating 50 Years

Prefrontal Coexpression of Schizophrenia Risk Genes Is Associated With Treatment Response in Patients

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ABSTRACT

BACKGROUND: Gene coexpression networks are relevant to functional and clinical translation of schizophrenia risk genes. We hypothesized that schizophrenia risk genes converge into coexpression pathways that may be associated with gene regulation mechanisms and with response to treatment in patients with schizophrenia.

METHODS: We identified gene coexpression networks in two prefrontal cortex postmortem RNA sequencing datasets (n = 688) and replicated them in four more datasets (n = 1295). We identified and replicated (p values $<$ .001) a single module enriched for schizophrenia risk loci (13 risk genes in 10 loci). In silico screening of potential regulators of the schizophrenia risk module via bioinformatic analyses identified two transcription factors and three microRNAs associated with the risk module. To translate postmortem information into clinical phenotypes, we identified polymorphisms predicting coexpression and combined them to obtain an index approximating module coexpression (Polygenic Coexpression Index [PCI]).

RESULTS: The PCI-coexpression association was successfully replicated in two independent brain transcriptome datasets (n = 131; p values $<$ .05). Finally, we tested the association between the PCI and short-term treatment response in two independent samples of patients with schizophrenia treated with olanzapine (n = 167). The PCI was associated with treatment response in the positive symptom domain in both clinical cohorts (p values $<$ .05).

CONCLUSIONS: In summary, our findings in 1983 samples of human postmortem prefrontal cortex show that coexpression of a set of genes enriched for schizophrenia risk genes is relevant to treatment response. This coexpression pathway may be coregulated by transcription factors and microRNA associated with it.

Keywords: Dorsolateral prefrontal cortex, Gene coexpression networks, Olanzapine, RNA sequencing, Schizophrenia

https://doi.org/10.1016/j.biopsych.2019.03.981

Risk for several major psychiatric disorders is highly related to genetic factors, and transcriptomics is leading the way to clarify how genetic risk translates into biological pathways underlying mental illness (1–3). This task is challenging because only rarely do we know which genes within the risk loci are causally implicated in the disorder (4). Psychiatric risk loci include many genes and are proximal to many more, such that risk variants in the loci may theoretically impact hundreds of genes (5). Understanding the relationship between risk variants and genes involved in the disorder may thus require identification of common pathways and biological processes involving genes located in multiple loci. In turn, discovering biological pathways that bring together multiple risk loci will contribute to identifying molecular elements, such as transcription factors and microRNA (miRNA), that may represent nodes of risk convergence by regulating diverse gene functions (6).

The novel insights from the analysis of postmortem brains are especially relevant for highly heritable disorders, such as schizophrenia (SCZ) (7–9). The discovery that at least 108 genetic loci are associated with the disease suggests that multiple biological processes may be involved in SCZ, perhaps converging into one or a few common pathways (high coherence) or distributed across many pathways of genetic risk (low coherence) (10). The question of genetic risk coherence is important because the functional and clinical translation of SCZ risk variants remains unclear when they are considered on their own or cumulated without links with the underlying biology. For example, currently available polygenic risk scores have explained no more than 1.2% of the variance in symptoms across patients (11–15) and up to 3.2% in treatment response or resistance (16–18).

A basic principle of biology is that the expression of individual genes is often coordinated by regulatory molecules, resulting in the coexpression of gene networks (1,6,19,20). Therefore, gene coexpression is a biological process

ISSN: 0006-3223

relevant to the convergence of psychiatric risk into common pathways that are associated with clinical translation of risk loci (3,21). At the very least, some of the SCZ risk variants control gene expression (22,23). Moreover, genes in the Psychiatric Genomics Consortium (PGC) SCZ loci cosegregate into coexpression pathways (3,21,22,24), and genetic variation in such pathways is relevant to SCZ phenotypes (2,25–28).

To the extent that risk gene expression underlies the symptoms that together characterize SCZ, risk gene expression patterns may also be related, with the response to drugs antagonizing such pathophysiological mechanisms. Indeed, the case of the dopamine receptor D2 gene (*DRD2*) is consistent with this hypothesis (7,25). Therefore, we hypothesized that genes located in PGC SCZ risk loci may converge into coexpression pathways that, in turn, reveal molecular elements that potentially contribute to orchestrate genetic risk and ultimately clinical outcome. It has never been investigated whether gene–gene relationships of relevance to SCZ risk are also relevant to interindividual differences between patients. Previous works translated coexpression pathways into predictions of drug response based on the individual genetic background associated with coexpression (25,29), but the association of such pathways with SCZ risk counted only a few genes.

Here, we aimed to identify, validate, and translate into clinical phenotypes gene coexpression networks (Figure 1) obtained by means of weighted gene coexpression network analysis (20,30). We used RNA sequencing data from two of the largest collections of postmortem prefrontal cortices currently available: the Lieber Institute for Brain Development (LIBD) repository (31) and the CommonMind Consortium (CMC) collection (22). We translated transcriptomics into clinical phenotypes using common genetic variants representing coexpression quantitative trait loci (co-eQTLs) (25,32). We associated co-eQTLs with short-term treatment response to olanzapine (assessed via Positive and Negative Syndrome Scale [PANSS]) in the largest double-blinded clinical trial openly available to date with genome-wide genotyping (33). We replicated the clinical results in an independent dataset of patients with SCZ treated with olanzapine in Bari, Italy (34).

METHODS AND MATERIALS

Coexpression Network of Human Prefrontal Cortex

Both the LIBD and CMC datasets included postmortem messenger RNA expression levels of healthy control subjects and patients with SCZ in the human prefrontal cortex, whereas three of the additional datasets used for replication included only individuals without diagnosed psychiatric disorders (35,36) and the fourth included participants with other major psychiatric disorders (21). We focused on this brain region because of the large sample size available and the multiple datasets usable for replication. The LIBD and CMC datasets were filtered based on RNA integrity number (≥7.0), age range (17–86 years), and ethnicity (African American and Caucasian). We included multiple ethnicities, even though risk loci have been identified in individuals with Caucasian ancestry (7), to maximize statistical power. After filtering, frontal cortex samples from 343 LIBD individuals and 345 CMC individuals were included (Supplemental Methods and Materials 1.1, 1.2). Supplemental Table S1 summarizes the demographic data of all samples.

We included genes available in both datasets with median expression >0.1 reads per kilobase per million (20,993). Based on the greater signal-to-noise ratio found in the LIBD dataset ($p < 2.2 \times 10^{-16}$) (Supplemental Methods and Materials 1.3 and Supplemental Results 2.1; Supplemental Figure S1), we selected the LIBD dataset as the reference and used the CMC dataset for replication. Thus, after preprocessing (37) (Supplemental Figures S2–S5), we identified one network for the LIBD and one for the CMC datasets, pooling data from patients and control subjects (Supplemental Methods and Materials 1.4, 1.5; Supplemental Results 2.2, 2.3). We also explored further samples to assess the robustness of the gene–gene relationships detected using four additional frontal cortex microarray and RNA sequencing datasets, as reported in Supplemental Table S1 (21,35,36,38). For this purpose, we employed preservation (39) and permutation (40) techniques (Supplemental Methods and Materials 1.6).

Prioritization of Modules Relevant for SCZ

We prioritized modules in terms of their overrepresentation of SCZ risk genes (n = 310; Supplemental Table S6) using

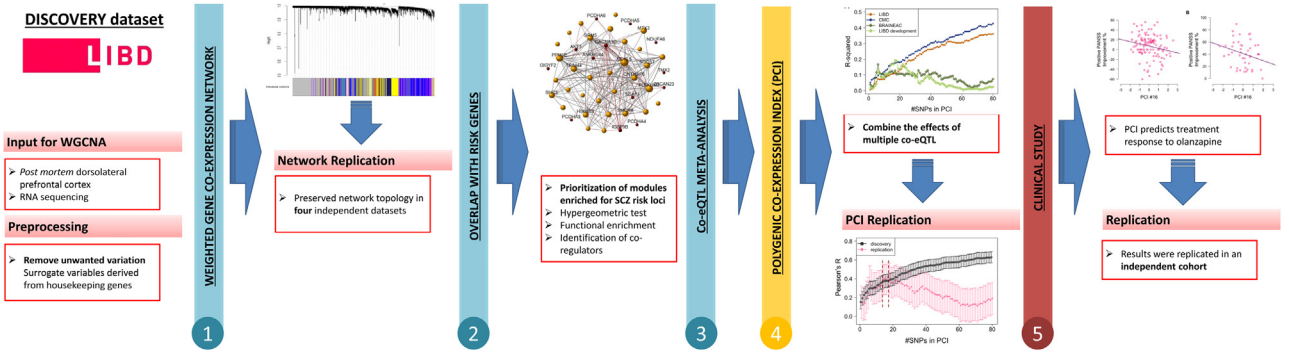

Figure 1. Study design. CMC, CommonMind Consortium; Co-eQTL, coexpression quantitative trait loci; LIBD, Lieber Institute for Brain Development; PANSS, Positive and Negative Syndrome Scale; PCI, Polygenic Coexpression Index; SCZ, schizophrenia; SNP, single nucleotide polymorphism; WGCNA, weighted gene coexpression network analysis.

hypergeometric tests and corrected the results for multiple comparisons (Bonferroni-corrected p value $<$ .05). We assessed overrepresentation in both protein-coding and non–protein-coding genes [*biomaRt* R package (41)]. As a negative control, we also tested the genome-wide association study loci for attention-deficit/hyperactivity disorder, autism spectrum disorder, bipolar disorder, and major depressive disorder (14,42,43) (Supplemental Methods and Materials 1.7). Next, we asked whether this enrichment was affected by genetic proximity. We hypothesized that the overrepresentation of SCZ risk genes remained significant when the boundaries of the loci were expanded within a genomic distance compatible with an influence of sequence elements on gene expression (44,45). To this end, we expanded SCZ risk loci from ±50 kbp to ±10 Mbp and derived an empirical p value via permutations (n = 10,000). At each iteration, we permuted the module assignment of each gene, we computed the hypergeometric probability for each set of random modules, and we retained the lowest p value to generate a null distribution. The empirical p value was defined as the rate of p values smaller than the threshold. We set the significance threshold at empirical p value $<$ .001.

Additionally, we computed a gene set competitive enrichment analysis with the software MAGMA (46) to assess whether variants that fell within enriched modules were associated with greater SCZ risk compared with risk in the remaining gene sets (Supplemental Methods and Materials 1.7). Finally, we tested whether the first principal component of module gene expression (module eigengene [ME]) was associated with possible biological confounders such as smoking habit or antipsychotic or antidepressant medications in patients with SCZ. To this aim, we used a binary classification of whether patients used the drugs (Supplemental Methods and Materials 1.5).

Functional Significance of Risk Modules

Given the importance of developmental ages for SCZ liability (47), we asked whether the genes in the risk modules were also coexpressed during neurodevelopment; to this end, we replicated our modules in a sample of 93 neurotypical individuals ranging in age from fetus to 16 years of age (LIBD developmental series; Supplemental Table S1) nonoverlapping with the sample used in the main analysis. Specific expression analysis (http://genetics.wustl.edu/jdlab/csea-tool-2/) (48) served to assess whether module genes were preferentially expressed in the cortex during specific neurodevelopmental stages (49) (Supplemental Figure S4). We also investigated module enrichment for brain cell type–specific markers (50) (Supplemental Methods and Materials 1.8). Then, we investigated the functional significance of the target modules by means of gene ontology, disease association, and chromosomal enrichment analyses [Amigo2; ToppGene (51)] (Supplemental Methods and Materials 1.8). We assessed whether medication in patients with SCZ may influence risk modules by investigating the overrepresentation of genes regulated by haloperidol (52) (Supplemental Methods and Materials 1.8). Finally, we assessed the overrepresentation of genes associated with SCZ from transcriptome-wide association studies (3,53) (Supplemental Methods and Materials 1.8).

Regulation of Transcription Potentially Implicated in Risk Module Coexpression

We hypothesized that coexpressed genes may be coregulated by elements such as transcription factors (TFs) and miRNA (54). We tested this hypothesis by investigating TFs targeting risk module genes. Using the software Pscan (http://159.149.160.88/pscan/) (55), we identified TFs whose binding motif was overrepresented in the promoter regions of our coexpressed genes (Bonferroni-corrected p value $<$ .05). We assessed all network modules to investigate the specificity of the associations. Additionally, we assessed the overrepresentation of the targetome of 10 miRNAs associated with SCZ risk (56) in all modules, using hypergeometric tests and permutations (Bonferroni-corrected p value $<$ .05) (Supplemental Methods and Materials 1.9).

Polygenic Coexpression Index

To translate risk module coexpression into clinical phenotypes, we generated an index predicting risk module coexpression based on individual genetic background. We first identified single nucleotide polymorphisms (SNPs) predicting coexpression (co-eQTLs) of the whole module and generated a Polygenic Coexpression Index (PCI) (25,32). We used a robust linear model to assess allelic dose effects on ME, adjusted for diagnosis, age, sex, RNA integrity number, the rate of reads mapped to known genes, the rate of reads mapped to mitochondrial DNA, and 10 genomic principal components accounting for population stratification. Aiming to increase statistical power, we computed a meta-analytic p value for each SNP based on the effect size in the LIBD and CMC datasets (meta-analytic dataset; overall, 688 participants; Supplemental Methods and Materials 1.10). We ranked SNPs based on their meta-analytic p value and computed several PCIs by adding one SNP at a time. Our purpose was to identify an ensemble of SNPs affording prediction of coexpression (correlation between ME and PCIs) rather than identifying single genetic variants associated with coexpression per se. To determine how many variants should be included in the PCI, we replicated the association between ME and PCIs in two independent datasets (BRAINEAC and LIBD developmental series, p value $<$ .05) (35,57) and aggregated the replication effect sizes through meta-analysis. The test sets did not affect the model because both the ME and the weights of the SNPs in the PCI were derived from the training sets (Supplemental Methods and Materials 1.11). We employed a previously published procedure based on signal detection theory to assign weights (A′) to each SNP genotype (25,32). The selected PCIs were used to predict treatment response to olanzapine in two cohorts of patients with SCZ.

Clinical Study

All participants provided written informed consent following the guidelines of the Declaration of Helsinki after receiving a complete description of the study. Protocols and procedures were approved by the ethics committee of the University of Bari and by the institutional review board of each clinical site involved in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) program. Diagnosis of SCZ was established via Structured Clinical Interview for DSM-IV-TR. Symptom severity was assessed with PANSS (58) at study entry and

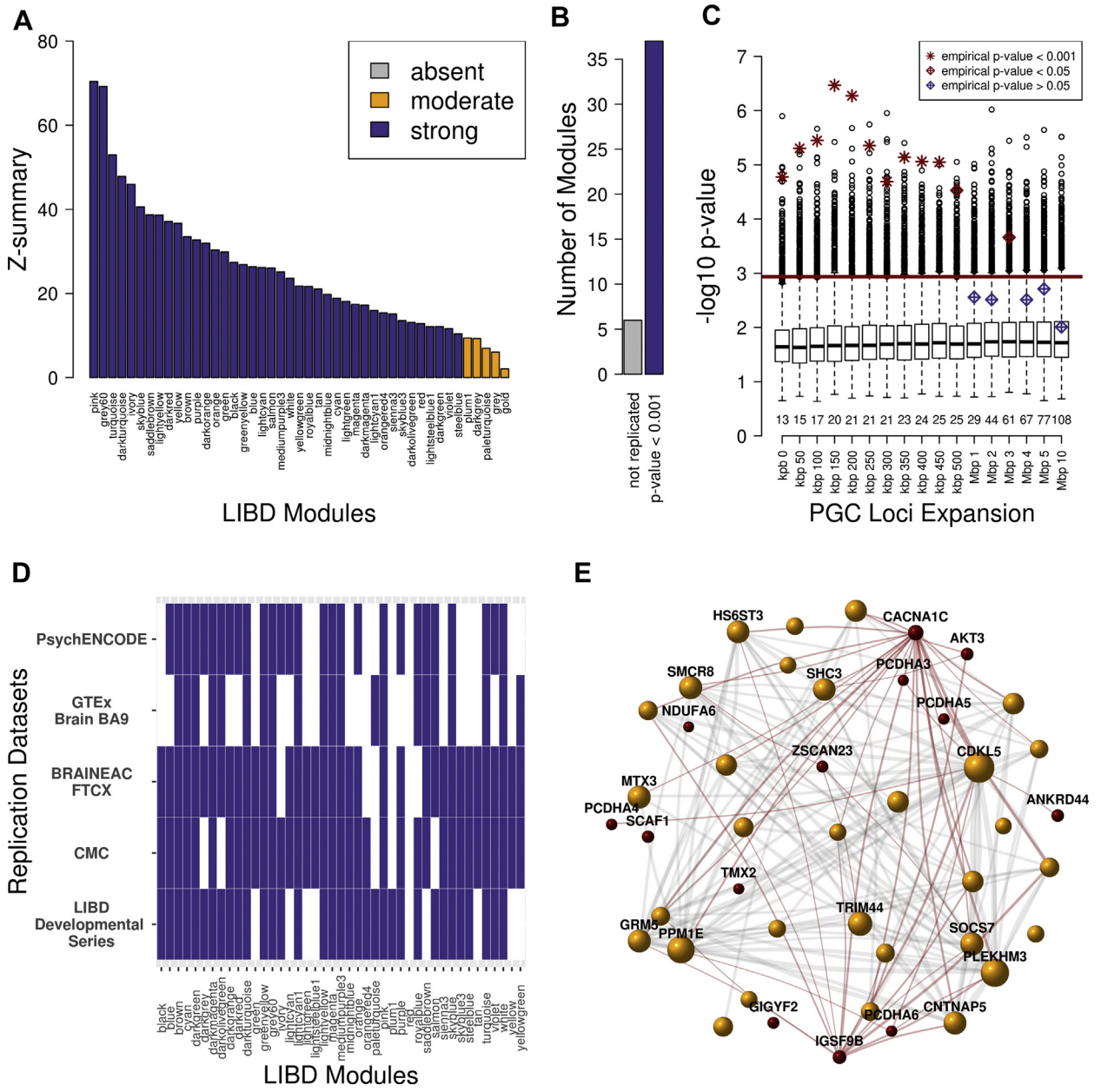

Figure 2. Coexpression network. **(A)** Preservation of the Lieber Institute for Brain Development (LIBD) network in the CommonMind Consortium (CMC) dataset (39). The LIBD modules are shown on the x-axis ranked by *Z*-summary preservation score (y-axis). $Z \geq 10$ denotes strong preservation, $2 \leq Z < 10$ moderate, and $Z < 2$ absent. **(B)** Replication of the LIBD modules topology in the CMC dataset (40). Bars indicate the number of replicated modules at empirical *p* value < .001 vs. not replicated modules (10,000 permutations). **(C)** *Darkgreen* module enrichment for schizophrenia (SCZ) risk genes. Enrichment significance is shown over increasing expansion of SCZ risk loci boundaries. The x-axis reports the size of expansion in kilobase pairs (kbp). The y-axis indicates the $-\log_{10} p$ value of the hypergeometric test for overrepresentation of SCZ risk loci in *Darkgreen*. Box plots show the null distribution of the lowest enrichment *p* value over all network modules obtained after network labels permutation (n = 10,000). The red horizontal line shows the Bonferroni threshold selected (number of modules = 43, α = .0012). Stars and diamonds denote *Darkgreen* exact enrichment *p* value. Numbers above the x-axis report the absolute number of SCZ risk genes found in *Darkgreen*. **(D)** Replication of the LIBD modules in several different datasets (40). Slate-blue fields denote modules (x-axis) replicated at empirical *p* value < .001 (over 10,000 permutations). **(E)** *Darkgreen* graph. The nodes of the graphs (spheres) represent genes, and SCZ risk genes are colored in dark red. Gold spheres represent a selection of the most connected genes in the module (hub genes, scaled intramodular connectivity ≥ 0.3) and have a diameter proportional to intramodular connectivity, i.e., larger spheres denote genes harboring more connections within *Darkgreen*. Lines denote gene–gene relationships and their width is proportional to connection strength. BA, Brodmann area; FTCX, frontal cortex; GTEx, Genotype-Tissue Expression Consortium; PGC, Psychiatric Genomics Consortium.

Table 1. Psychiatric Genomic Consortium (PGC) Loci and Genes Overlapping With the Module *Darkgreen*

Ensembl Gene ID	HGNC Symbol	Gene Name	PGC Loci Rank	PGC Loci Index SNP	PGC Loci Position (hg19[a])	PGC Index SNP *p* Value
ENSG00000187987	*ZSCAN23*	Zinc finger and SCAN domain containing 23	1	rs115329265	chr6:28303247–28712247	3.48×10^{-31}
ENSG00000151067	*CACNA1C*	Calcium voltage-gated channel subunit alpha1 C	4	rs2007044, rs2239063	chr12:2321860–2523731	3.22×10^{-18}
ENSG00000204120	*GIGYF2*	GRB10 interacting GYF protein 2	22	rs6704768	chr2:233559301–233753501	2.32×10^{-12}
ENSG00000065413	*ANKRD44*	Ankyrin repeat domain 44	31	rs6434928	chr2:198148577–198835577	2.06×10^{-11}
ENSG00000080854	*IGSF9B*	Immunoglobulin superfamily, member 9B	36	rs75059851	chr11:133808069–133852969	3.87×10^{-11}
ENSG00000184983	*NDUFA6*	NADH:ubiquinone oxidoreductase subunit A6	57	rs1023500, rs6002655	chr22:42315744–42689414	1.71×10^{-9}
ENSG00000213593	*TMX2*	Thioredoxin-related transmembrane protein 2	59	rs9420	chr11:57386294–57682294	2.24×10^{-9}
ENSG00000117020	*AKT3*	AKT serine/threonine kinase 3	64	rs10803138, rs77149735, rs14403, chr1_243881945_I	chr1:243503719–244002945	3.73×10^{-9}
ENSG00000126461	*SCAF1*	SR-related CTD associated factor 1	106	rs56873913	chr19:50067499–50135399	4.69×10^{-8}
ENSG00000255408	*PCDHA3*	Protocadherin alpha 3	108	chr5_140143664_I	chr5:140023664–140222664	4.85×10^{-8}
ENSG00000204967	*PCDHA4*	Protocadherin alpha 4				
ENSG00000204965	*PCDHA5*	Protocadherin alpha 5				
ENSG00000081842	*PCDHA6*	Protocadherin alpha 6				

chr, chromosome; HGNC, HUGO Gene Nomenclature Committee; SNP, single nucleotide polymorphism.
[a]Human Genome version 19.

at several follow-up visits. We focused on patients treated with olanzapine because it showed the best response in the CATIE study (33) and because we had a replication sample available undergoing the same treatment. The first clinical cohort included patients recruited in the CATIE study by the National Institute of Mental Health and treated with olanzapine (n = 121) (33). The second cohort included 46 patients recruited from the region of Apulia, Italy, who were also treated with olanzapine in monotherapy (34) (Supplemental Methods and Materials 1.12).

We computed percent change of symptom severity from baseline to 1-month follow-up separately in the positive, negative, and general subscales of the PANSS, in both the CATIE and University of Bari datasets. Supplemental Table S2 summarizes PANSS scores. We used robust multiple regression to assess the association with the PCIs, controlling for age, sex, education level, and ancestry (indexed using the first 10 genomic principal components). We corrected statistics for multiple comparisons using p values adjusted for corrected tests, a method that identifies the number of statistically independent tests based on the correlation structure of the variables tested. We tested three clinical scores and four correlated PCIs and corrected for multiple comparisons after determining the interdependence between variables (corrected p value $< .05$) (59). To investigate the biological relevance of the SNP set predicting treatment outcome, we assessed the enrichment for genetic regulatory elements by interrogating Haploreg version 4.1 (Bonferroni-corrected p value $< .05$), a repository of previous genomic studies (60,61), including specific information on the dorsolateral prefrontal cortex (Supplemental Methods and Materials 1.11).

RESULTS

Coexpression Modules of Risk Genes for Schizophrenia

The LIBD network (available in Data File S1 in Supplement 2) was preserved in the CMC network, and vice versa (Figure 2A, 2B; Supplemental Figure S7; Supplemental Results 2.3; Supplemental Tables S3, S4). Across both datasets, we identified a single module (*Darkgreen*) (Figure 2E) significantly enriched for genes in the PGC SCZ loci (10 loci, 13 genes, Bonferroni-corrected p value over all modules generated across both networks = 3.1×10^{-3} (Table 1, Supplemental Table S7). No other associations of any of the modules with other psychiatric disorders were significant at the same threshold. Notably, the SCZ enrichment persisted when we included both protein-coding and non–protein-coding genes located in the PGC loci (p value = 5.7×10^{-4}) (Supplemental Figure S13) and when we controlled for guanine-cytosine content and gene length (Supplemental Results 2.4, Supplemental Figure S14). Interestingly, the module included *CACNA1C*, potassium channels, protocadherins, and several genes coding for neurotransmitter receptors. The enrichment survived permutation-based empirical p value $< .001$ when loci were

expanded up to 450 kbp (Figure 2C; Supplemental Figure S13). *Darkgreen* was among 13 modules preserved and topologically replicated in all of the four additional transcriptomic datasets we analyzed, showing that the identified gene–gene associations were robust (empirical p value $<$ .001) (Figure 2D; Supplemental Table S8). MAGMA (46) demonstrated that variants falling within *Darkgreen* were associated with greater SCZ risk compared with the remaining sets (p value = .036). Hence, converging evidence from the gene list and the localization of genetic variants suggested that genetic risk for SCZ converged into *Darkgreen*. Moreover, ME *Darkgreen* was not associated with smoking habit nor with use of antipsychotic or antidepressant medications in patients with SCZ (p value $>$ .1) (Data File S2 in Supplement 2).

Functional Significance of *Darkgreen* Module

Darkgreen included 225 genes (157 protein coding) (Supplemental Table S7). The LIBD developmental series revealed significant preservation and topological overlap (empirical p value $<$ .001; Supplemental Table S8), showing that *Darkgreen* gene–gene relationships were significant also in independent participants during developmental life stages. *Darkgreen* was enriched for genes preferentially expressed in the cortex during young adulthood (49) (Supplemental Figure S15) and for neuronal cell types with demonstrated association with SCZ risk (50) (Supplemental Results 2.5; Supplemental Figures S16, S17). *Darkgreen* was functionally enriched for gene products involved in homophilic cell adhesion via plasma membrane (Amigo2, GO:0007156, 9 genes, fold-enrichment = 7.92, Bonferroni-corrected p value = .022). Protocadherin genes located in the 5q31.3 region of chromosome 5 were overrepresented in *Darkgreen* (false discovery rate–corrected p value = 2.7×10^{-4}), whereas genes associated with haloperidol administration were not (Supplemental Results 2.5; Supplemental Figure S18). We found no significant enrichment for SCZ transcriptome-wide association in any coexpression module.

Regulation of Transcription Potentially Implicated in *Darkgreen* Module Coexpression

Pscan revealed two TFs (NRF1, KLF14) whose binding motif was overrepresented in the promoter regions of *Darkgreen*-coexpressed genes (corrected p value $<$ .05). However, the identified TFs were related to several other modules (corrected p value $<$ .05; NRF1 to 24 modules; KLF14 to 18 modules) (Supplemental Figure S19), hindering conclusions about their specificity. The targets of three SCZ-related miRNAs (miR-101, miR-374, and miR-28) were overrepresented in *Darkgreen* (corrected p value $<$ .05) (Supplemental Table S9). Interestingly, miR-374 targets eight of the nine *Darkgreen* genes with GO:0007156 (fold-enrichment = 21.5, Bonferroni-corrected p value = 3.8×10^{-5}). The identified miRNAs overlapped with only a few modules (miR-101, miR-374, and miR-28 overlapped with 8, 6, and 10 modules, respectively; corrected p value $<$ .05) (Supplemental Figure S20; Data file S3 in Supplement 2), suggesting some degree of specificity. Overall, these results are consistent with the idea that genetic risk convergence in *Darkgreen* may be mediated by TFs and miRNAs.

Polygenic Coexpression Index

The SNP association analysis revealed one SNP, rs9836592, surviving Bonferroni correction for multiple comparisons; however, variants significant at uncorrected thresholds added further predictive power to this single SNP, because PCIs including between six and 32 SNPs afforded significant predictive capacity in both datasets with an effect size comparable between discovery and replication sets (BRAINEAC: p value $<$ .05 [Figure 3A, 3B; Supplemental Figure S22]; LIBD developmental series: p value $<$ .05 [Figure 3A; Supplemental Figure S23]). Supplemental Table S10 includes annotations of the first 32 SNPs. In the meta-analysis of the BRAINEAC and the LIBD developmental series, prediction strength reached a plateau between 14 and 17 SNPs, with no further improvement when more SNPs were added (Figure 3C). Based on these results, we used the PCIs that included 14 to 17 SNPs as predictors in the clinical study.

Clinical Study

Table 2 illustrates the results of our clinical study. We found the most significant relationship between the PCI-16 and positive PANSS improvement (corrected p value = .033, η_p^2 = .061; estimated number of independent comparisons $\cong$ 4.66) (Figure 4A), which was replicated in the University of Bari independent clinical sample (one-tailed p = .0475, η_p^2 = .067) (Figure 4B). This set of 16 SNPs was enriched for H3K27ac-H3K9ac marks specifically in the dorsolateral prefrontal cortex (corrected p value = .029).

DISCUSSION

We investigated the coherence of SCZ PGC loci with the aim of identifying a biological pathway of SCZ risk associated with interindividual variation between patients. We identified one gene coexpression module enriched for genes located in risk loci for SCZ. Gene–gene relationships were reproducible in five other brain gene expression datasets. Co-eQTLs identified in 688 participants were associated with short-term treatment response to olanzapine—a first-line antipsychotic—in patients with SCZ. These findings suggest a significant degree of coherence of SCZ risk genes and coexpression partners that translates into interindividual variability in treatment response to olanzapine between patients.

Gene Coexpression in Schizophrenia

In the context of noncoding variation, which in genome-wide association studies characterizes most significant SNPs in common disorders, gene expression is likely the phenotype closest to DNA in which interindividual differences can be directly associated with genetic variation. The multifold preservation of the network is important to demonstrate that state-related factors such as pharmacological treatment did not dominate the topology of the network, which was replicated in independent datasets of individuals of various ages and affected by various psychiatric disorders totaling 1295 participants. Therefore, our results can be generalized beyond the discovery sample that includes healthy control subjects and

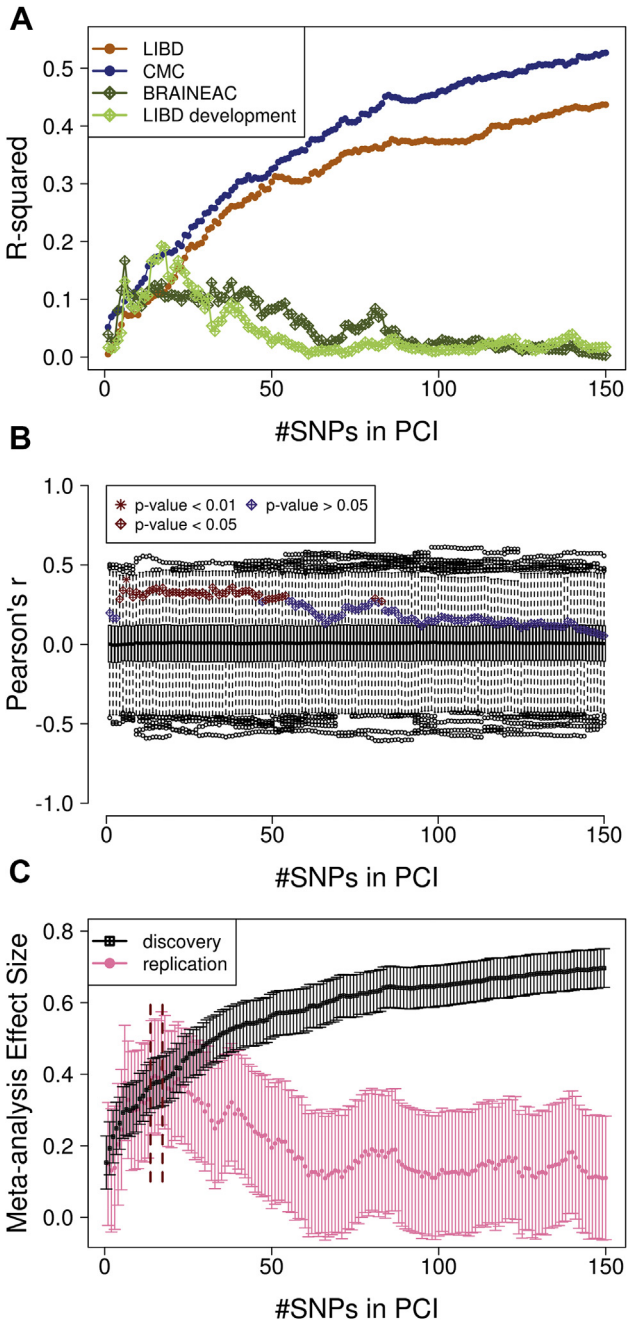

Figure 3. Polygenic Coexpression Index (PCI). **(A)** The plot illustrates the variation of the effect size of the correlation between the PCI and the *Darkgreen* module eigengene (ME) (y-axis) for a series of PCIs with incrementally added single nucleotide polymorphisms (SNPs). The discovery (Lieber Institute for Brain Development [LIBD], CommonMind Consortium [CMC]) and replication datasets (BRAINEAC, LIBD development) are represented with different colors. For increasing number of SNPs included in the PCI (x-axis), the effect size in the discovery sets increases monotonically because of overfitting, while it remains stable and then drops in the replication set, suggesting an optimal signal-to-noise ratio in the replication set between six and about 32 SNPs. **(B)** PCI replication. Empirical significance of the correlations between PCIs and ME in the replication set (BRAINEAC). Stars and diamonds display on the y-axis the significance of each ME–PCI correlation over an increasing number of SNPs (x-axis). Box plots show the corresponding null distribution of the correlation coefficients when genotypes are permuted (2000 permutations). Color and shape key in the panel highlight different empirical significance cut-offs. **(C)** Meta-analysis of the effect sizes (Pearson's *r*) in the discovery and replication datasets. Dark red vertical dashed lines delimit a plateau in the replication effect sizes between 14 and 17 SNPs. Note that the effect size never increases above the level observed at the 17th SNP.

Table 2. Association Between Polygenic Coexpression Indices (PCIs) and Positive Early Treatment Response on the Positive and Negative Syndrome Scale (PANSS)

PANSS Subscales	SNPs in the PCI, No.	CATIE			UNIBA	
		t Value	*p* Value	Corrected *p* Value	*t* Value	One-sided *p* Value
Positive	PCI #14	−2.11	.03681	.134	−1.93	.0306
	PCI #15	−2.74	.00714	.033	−1.79	.0403
	PCI #16	−2.74	.00708	.033	−1.71	.0475
	PCI #17	−2.58	.01119	.049	−1.60	.0553
Negative	PCI #14	0.59	.5575	1	1.14	.1311
	PCI #15	0.29	.7750	1	1.18	.1218
	PCI #16	0.28	.7816	1	1.35	.0925
	PCI #17	0.22	.8198	1	1.42	.0817
General	PCI #14	−1.52	.1301	.277	−0.28	.3889
	PCI #15	−1.81	.0721	.191	−0.19	.4234
	PCI #16	−1.74	.0837	.208	−0.24	.4075
	PCI #17	−1.78	.0771	.198	−0.23	.4106

CATIE, Clinical Antipsychotic Trials of Intervention Effectiveness; SNP, single nucleotide polymorphism; UNIBA, University of Bari.

patients with SCZ. Consistently, gene–gene relationships within *Darkgreen* were not associated with smoking or antipsychotic medication, as far as these phenomenological factors are measurable in postmortem tissue. Furthermore, the possible confounding effects of medication may depend on the specific antipsychotic administered and on the dose. In spite of different acquisition and processing pipelines across the multiple datasets we used, the replicated results highlight that findings are not bound to specific parameters (62). In summary, we identified gene–gene relationships revealing coherence of genetic risk for SCZ across 10 separate genomic loci.

The Schizophrenia Risk Coexpression Module

Previous reports on gene coexpression in the prefrontal cortex highlighted modules enriched for SCZ risk variants, as well as for risk genes (24,28,63) and for differentially expressed genes (3). The module we identified shows overrepresentation of risk SNPs and risk genes at multiple genomic distance thresholds up to 450 kbp, thus implicating genes beyond those initially reported as associated with SCZ (7) and converging from 10 different loci. Enrichment analyses revealed an involvement of *Darkgreen* genes in cell–cell adhesion, especially mediated by the SCZ risk locus including several protocadherin genes—a biological process previously associated with risk for SCZ and bipolar disorder (64,65). Although we selected genes expressed in the brain, ontologies were not filtered in the same way; hence, more biological functions than currently detected may be shared by these genes. Interestingly, the same protocadherins

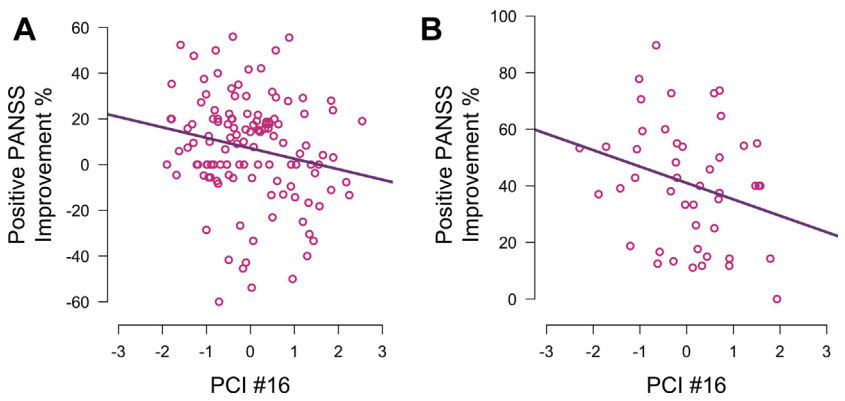

Figure 4. Association between the Polygenic Coexpression Index (PCI) and clinical outcome. Negative correlation was found between the PCI with 16 single nucleotide polymorphisms and symptom improvement in the positive domain of the Positive and Negative Syndrome Scale (PANSS) (difference between end point and baseline relative to baseline, shown on the y-axis) in the **(A)** Clinical Antipsychotic Trials of Intervention Effectiveness and **(B)** University of Bari cohorts.

were found to be associated with SCZ in induced pluripotent stem cell–derived differentiated neurons (66) and with treatment response in monozygotic twins with discordant response to clozapine in treatment-resistant SCZ (67). Taken together, these findings highlight the potential importance of *Darkgreen* genes for the physiology of olanzapine and clozapine, two atypical antipsychotics. *Darkgreen* also included genes coding for proteins involved in synaptic transmission mediated by serotonin, glutamate, and gamma-aminobutyric acid (*HTR1F*, *GRM5*, *GABRB1*, *GABRG3*) and for those involved in neural excitability (*KCNH1*, *KCNA3*, *KCNH7*, *KCNH5*), along with *CACNA1C*, a risk gene for SCZ and bipolar disorder supported by multiple lines of evidence (68–72) and previously associated with response to olanzapine (73). The functions of the genes in *Darkgreen* are consistent with previous pathway analyses of SCZ risk (74) and enhance the biological plausibility of the functional relevance of *Darkgreen* coregulation.

Although coexpression is only suggestive of gene coregulation, it is noteworthy that the 13 PGC hits of *Darkgreen* are distributed across 10 different loci, rather than encompassing a single locus that is cotranscripted because of genetic proximity (44,45). This finding suggests that there may be coregulators of these 10 loci, which we attempted to identify via bioinformatics. Further investigations are required to clarify the link between these gene regulators, the coexpressed SCZ risk genes we identified, and neurodevelopment, a link suggested by the significant preservation of *Darkgreen* topology in very young participants and by prior reports (75–77).

Genetic Variants Associated With Coexpression of Schizophrenia Risk Genes

As it is not possible to directly assess gene expression in the living human brain, we used postmortem data to weight co-eQTL alleles with the aim to predict the shared variance between coexpressed genes and hence index coexpression in living individuals. The co-eQTLs detected here merit further investigation as potential indicators of loci affected by genetic regulatory elements associated with positive symptoms and their clinical course. For example, the first ranked SNP, rs9836592, has been associated with risk for bipolar disorder (78), another disorder frequently treated with antipsychotic drugs such as olanzapine. Furthermore, this SNP has been already associated with the regulation of gene expression (78) and is an eQTL for *CACNA1D* (79). Moreover, our set of 16 SNPs was enriched for histone acetylation marks. Previous evidence supports the relevance of histone modification pathways to SCZ (74) and the specific role of H3K27ac markers in autism (80) a neurodevelopmental disorder sharing some genetic risk with SCZ (81).

Response to Treatment With Antipsychotics

The PCI that was computed using the above-described genetic variants was reproducibly associated with treatment response to olanzapine. On the one hand, this finding suggests that the 13 PGC hit genes coexpressed in *Darkgreen* are candidates within their loci for mechanistic interpretations of response to treatment. On the other hand, the PCI indexes a wider group of genes, going beyond the PGC hits, suggesting a broader transcriptomic landscape of risk and, more relevant here, of the biology of treatment response. The present findings imply that antipsychotic efficacy may involve many more genes than those coding for the traditional targets, e.g., dopamine (25,29) and serotonin antagonism, tapping into multiple neural transmission systems, including glutamate and gamma-aminobutyric acid receptors, and calcium and potassium channels.

Our findings further suggest a link of SCZ risk loci and their molecular interactors with interindividual variation in response to treatment with olanzapine selectively for positive symptoms domain. As the two datasets we considered differed in overall treatment response, it is not possible to determine a PCI cutoff associated with a given threshold of treatment response. The current evidence is also limited by the relatively restricted sample size in the clinical groups (n = 167) and by the modest size of the treatment-response effects, although 6% of the variance explained compares favorably with prior reports of SCZ polygenic risk (16–18). Therefore, these results appear promising with respect to the feasibility of patient stratification based on biological measures, in line with dimensional views of SCZ (82–86).

Some limitations of this study suggest caution. While weighted gene coexpression network analysis is a flexible and extensively used tool, gene–gene relationships can be reflected in different gene clustering across datasets and

studies. Gene–gene relationships may also depend on confounding variables such as ethnicity. The analyses we performed to control for confounders did not reveal a significant impact of population stratification on our findings. Furthermore, the role of potential regulators of gene coexpression requires biological evidence to offer mechanistic explanations of their relationships with response to olanzapine. Nevertheless, this work and further evidence (3,21,22,24) demonstrate the coherence of a subset of SCZ risk genes mediated by coexpression. Our PCI approach was aimed at studying the interindividual variability across patients rather than between patients and healthy control subjects. The findings offer the first proof of concept that the genome-wide significant convergence of SCZ risk genes in specific coexpression modules translates into interindividual variability of treatment response in patients.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by a "Capitale Umano ad Alta Qualificazione" grant by Fondazione Con Il Sud (to AB), by the National Association for Research on Schizophrenia and Affective Disorders (Grant No. 28935 [to AB]), and by the "Ricerca Finalizzata" (Grant No. PE-2011–02347951 [to AB]); by the Lieber Institute for Brain Development (to DRW); and by a Hoffmann-La Roche Collaboration Grant (to GP). This project has received funding from the European Union Seventh Framework Programme for research, technological development and demonstration (Grant No. 602450) (IMAGEMEND [to AB]). GP's position is funded by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant (Grant No. 798181 [to GP]) (FLOURISH). This article reflects only the authors' views, and the European Union and Research Executive Agency are not liable for any use that may be made of the information contained therein.

GP, PDC, DRW, and AB designed the study; TMH, JEK, JHS, GB, DRW, AR, and AB were involved in data collection; PDC, AEJ, MP, JHS, and QC analyzed the data; GP, PDC, AEJ, DRW, and AB interpreted the data; GP, PDC, and AB wrote the first draft of the manuscript; all authors revised and approved the manuscript.

This work complements further reports on partially overlapping datasets that focused on network approaches to identify potential novel drug targets (24). This article was based on results from the CATIE project supported with federal funds from the National Institute of Mental Health (NIMH) under contract NO1 MH90001. The project was carried out by principal investigators from the University of North Carolina, Duke University, the University of Southern California, the University of Rochester, and Yale University in association with Quintiles, Inc., and the program staff of the Division of Interventions and Services Research of the NIMH and investigators from 84 sites in the United States. AstraZeneca Pharmaceuticals, Bristol-Myers Squibb Company, Forest Pharmaceuticals, Janssen Pharmaceutica Products, Eli Lilly and Company, Otsuka Pharmaceutical Company, Pfizer, and Zenith Goldline Pharmaceuticals provided medications for the studies. CMC data were generously provided to GP by the NIMH and CommonMind Consortium. We gratefully acknowledge the work by Prof. Roberto Bellotti, Dr. Alfonso Monaco (Department of Physics–University of Bari Aldo Moro) and Dr. Piergiuseppe Di Palo, Marco Zezza, Leonardo Sportelli, Andrea Gaudio, and Elisabetta Volpe (Department of Basic Medical Science, Neuroscience, and Sense Organs–University of Bari Aldo Moro), who contributed to data analysis. We are also in debt to Dr. Gianluca Ursini, Dr. Richard Straub, and Dr. Venkata S. Mattay (Lieber Institute for Brain Development) for insightful discussions on the procedures employed.

AB has received consulting fees from Biogen and lecture fees from Otsuka, Janssen, and Lundbeck. GP has been the academic supervisor of a Roche collaboration grant (years 2015–2016) that funded his and AR's salary. AR has received travel fees from Lundbeck. All other authors report no biomedical financial interests or potential conflicts of interest.

Codes for the main analyses are available at: https://github.com/pdicarl3/BioIPsychiatry_2019.

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Received Jun 27, 2018; revised Mar 13, 2019; accepted Mar 14, 2019.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2019.03.981.

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