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Genome-wide gene pathway analysis of psychotic illness symptom dimensions based on a new schizophrenia-specific model of the OPCRIT

Anna R. Docherty^{1,*}, T. Bernard Bigdeli¹, Alexis C. Edwards¹, Silviu Bacanu¹, Donghyung Lee¹, Michael C. Neale¹, Brandon K. Wormley¹, Dermot Walsh², F. Anthony O'Neill³, Brien P. Riley¹, Kenneth S. Kendler¹, and Ayman H. Fanous^{1,4,5}

¹Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University School of Medicine, Virginia, USA ²Queen's University, Belfast, Ireland ³Center for Public Health, Belfast, Ireland ⁴Washington Veterans Affairs Healthcare System, Washington D.C. USA ⁵Georgetown University School of Medicine, Washington D.C. USA

Abstract

Empirically derived phenotypic measurements have the potential to enhance gene-finding efforts in schizophrenia. Previous research based on factor analyses of symptoms has typically included schizoaffective cases. Deriving factor loadings from analysis of only narrowly defined schizophrenia cases could yield more sensitive factor scores for gene pathway and gene ontology analyses. Using an Irish family sample, this study 1) factor analyzed clinician-rated Operational Criteria Checklist items in cases with schizophrenia only, 2) scored the full sample based on these factor loadings, and 3) implemented genome-wide association, gene-based, and gene-pathway analysis of these SCZ-based symptom factors (final N = 507). Three factors emerged from the analysis of the schizophrenia cases: a manic, a depressive, and a positive symptom factor. In gene-based analyses of these factors, multiple genes had $q < 0.01$. Of particular interest are findings for *PTPRG* and *WBPIL*, both of which were previously implicated by the Psychiatric Genomics Consortium study of SCZ; results from this study suggest that variants in these genes might also act as modifiers of SCZ symptoms. Gene pathway analyses of the first factor indicated over-representation of glutamatergic transmission, GABA-A receptor, and cyclic GMP pathways.

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*Corresponding author (Permanent address): Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University School of Medicine, 1P-132 Biotech One, 800 East Leigh Street, Richmond, VA 23220, USA, tel. +1 804 828 8127, fax. +1 804 828 1471, ardocherty@vcu.edu.

Conflict of Interest

The authors have no conflicts of interest to report.

Contributors

AD planned the analysis, managed the literature searches, undertook the statistical analyses, and wrote the first draft of the manuscript. KK, AF, DW, FO, and BR designed the data collection protocol and provided funding for the study. AF, TB, and AE provided statistical expertise and commentary on drafts of the manuscript. SB, DL, TB, and BR provided quality control, SNP imputation methods, and DNA processing. All authors contributed to and have approved the final manuscript.

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Results suggest that these pathways may have differential influence on affective symptom presentation in schizophrenia.

Keywords

genetic; OPCRIT; schizophrenia; dimensional assessment; symptoms; modifier gene; gene pathway; gene enrichment

1. Introduction

Enhancing phenotype measurement could aid in the identification of genes that modify schizophrenia symptoms. Research has moved toward a dimensional rather than categorical approach to understanding the genetics of schizophrenia (SCZ), by using continuous symptom phenotypes rather than simply dichotomous, case-control status (e.g., Fanous et al, 2008, 2012; Derks et al., 2012; Ruderfer et al., 2014). This approach, consistent with NIMH's Research Domain Criteria initiative (e.g., Insel et al., 2010), is more sensitive to subtle variation in the phenotype, and might increase power to detect genes and gene pathways involved in SCZ symptoms.

Following some evidence for genetic linkage to particular chromosomal regions for illness subtypes (Fanous et al., 2008) we recently examined SCZ symptom domains (positive and negative symptoms) by genome-wide association (GWA) to attempt to account for some of the clinical heterogeneity in SCZ (Fanous et al., 2012; Edwards et al., in press). This research used quantitative phenotypes based on factor analyses of both affective and non-affective cases with psychosis. Symptoms were associated with gene pathways involving a broad range of functions (e.g., addiction, immune functioning) using this dimensional approach to identifying modifier genes.

A key feature of these analyses was that core negative symptoms such as anhedonia tended to load on a depression factor. However, anhedonia is a classical negative symptom of SCZ (Kraepelin, 1919; Bleuler, 1950; Rado, 1953). It remains unclear to what extent the symptoms shared between SCZ and affective disorders, such as anhedonia, arise from distinct pathophysiological mechanisms. It is possible that joint factor analysis of affective and non-affective psychosis cases could reveal a factor structure emphasizing affect as a disproportionately separate "domain" from other, classical SCZ symptoms. Similarly, symptoms such as grandiose delusions may disproportionately load on a mania factor, but not a positive symptom factor, in analyses that include individuals with both SCZ and affective disorders.

We sought to be agnostic with respect to the interpretation of SCZ symptom factors in this study and to focus solely on the "core" illness as the basis for factor loadings. In this study, we hypothesized that including only SCZ cases in a factor analysis of the Operational Criteria Checklist for Psychotic Disorders (OPCRIT; McGuffin et al., 1991) would yield loadings more specific to the SCZ spectrum.

We also predicted that such factors would be more sensitive to detecting associations with gene pathways or ontologies specific to SCZ. Using an Irish pedigree sample (Kendler et al., 1996), this study sought to 1) factor analyze only cases with a diagnosis of SCZ while excluding schizoaffective disorder and psychotic mood disorders, 2) score the full sample of cases based on these factor loadings, and 3) implement genome-wide association analyses, gene-based analyses, and gene pathway analyses of each of these putatively SCZ-specific symptom factors. We hypothesized that because factors are based on symptoms in narrowly-defined schizophrenia cases, factor scores would be sensitive to gene pathway enrichment related to SCZ neurotransmission.

2. Materials and Methods

2.1 Participant Ascertainment

This study examined a sample of over 800 cases with a history of affective or non-affective psychosis, from the Irish Study of High-Density Schizophrenia Families (Kendler et al., 1996). Ascertainment methods have been detailed elsewhere (Kendler et al., 1996) but briefly, structured clinical interview, medical records, and established consensus methods were used to determine psychiatric diagnosis and to make OPCRIT ratings. The OPCRIT is a comprehensive lifetime symptom scale based on assessment by trained clinicians.

2.2 Factor analysis

In this sample, ratings were completed after the clinician's administration of a structured diagnostic interview protocol. We conducted an exploratory factor analysis, in only cases with schizophrenia, of the clinician-rated OPCRIT items (listed in Supplement 1) using an oblique geomin rotation and R statistical software. Scores for the entire case sample, including all schizophrenia-spectrum disorders, were then calculated using weighted sums, and factor scores were then merged with available genetic data (final N = 507). The optimal number of factors was determined with a parallel analysis using the nFactors package for R (Raiche, 2011).

2.3 Genotyping and genome-wide association analyses

The sample was genotyped on the Illumina 610-Quad platform and was imputed to the publicly available 1000 Genomes reference panel. Detailed methods are available in (Bigdeli et al., 2014). Genome-wide, family-based association score tests of each of the symptom factors were conducted using the `-fastassoc` function (Chen & Abecasis, 2007) in MERLIN-OFFLINE (Abecasis et al., 2002) on SNPs with minor allele frequency > 1%. Manhattan plots of the symptom factor GWAS are presented in Figure 1.

2.4 Gene-based analyses

KGG 3.0 (Li et al., 2012) was used to complete gene-based analyses on each of the symptom factors. Differences in gene size and linkage were accounted for by correcting for linkage disequilibrium (LD) using the publicly available 1000 Genomes Phase 1 Version 3 (European subsample) LD files. We included extended gene lengths of 5kb at both the 5' and 3' ends. ($r^2 > .9$) SNPs in high LD with each other were considered to be connected, while those in low LD ($r^2 < .02$) were considered independent. We used the HYST test

option, which combines the extended Simes' and scaled chi-square tests. A Benjamini & Hochberg (1995) FDR of 0.05 was used, and q-values are reported where appropriate.

2.5 Gene pathway and gene ontology analyses

We annotated the SNP files including SNPs with p values < 0.001 using Plink (Purcell et al., 2007) and derived gene lists. We then uploaded the lists for enrichment analysis, within each separate symptom factor, to ConsensusPathDB (Kamburov et al., 2013). Gene ontology categories reflect groups of genes with functional commonalities. Each gene can belong to multiple categories, nested by hierarchical functional categories (e.g., drug binding is a "child" category of the more general category of "binding"). SNPs were mapped to genes, or to regions within 20kb of genes, using Plink annotation. A list of non-redundant genes was then uploaded to ConsensusPathDB. Pathway-based sets for each phenotype were defined by the following pathway databases--Manual upload, Signalink, Netpath, Kegg, Biocarta, Pharmgkb, and Reactome--at a stringent cutoff of $p < 0.001$. Gene ontology categories included levels 2 and 3, again at a stringent p-value cutoff of $p < 0.001$.

3. Results

3.1 Factor Analysis

Three factors emerged from the analysis of symptom ratings of the SCZ cases. Factor loadings are presented in Supplement 1. In these analyses, affective symptoms were parsed across the first two factors. Factor 1 comprised a mix of positively loading manic symptoms, hereafter called MAN, and many classic depressive symptoms positively loading on factor 2 (DEP). The discrimination between factors 1 and 2 result from rather strong positive loadings of the former on manic/agitated symptoms, suggesting that mood symptoms are prevalent in the cases with schizophrenia diagnoses. Schneiderian first-rank and other positive symptoms loaded (positively) almost exclusively on a third factor (POS). The first two factors were significantly positively correlated with each other ($r = 0.61$, $p < .001$). However, neither of these were significantly correlated with the third, POS factor ($r = -0.08$ and $r = -0.09$, respectively).

3.2 Genotyping and genome-wide association analyses

There was no obvious evidence of systematic inflation of genome-wide test statistics, as assessed by genomic inflation factor (factor 1 $\lambda = 1.03$, factor 2 $\lambda = 0.97$, factor 3 $\lambda = 1.00$). We observed no SNPs that were significant genome-wide for any of the factors. Suggestive associations ($p < 10^{-5}$) were observed for 196 SNPs (31 MAN; 103 DEP; 51 POS). We observed the strongest evidence of association between the first factor and an intronic variant in *DOK7* (rs143074317; $p = 2.79 \times 10^{-7}$), a gene essential for neuromuscular synaptogenesis.

3.3 Gene-based analyses

In gene-based analyses, a total of 9 genes across the three factors had $q < 0.01$. A summary of the genes with gene-based q values < 0.01 are presented in Table 1. *PTPRG* and *WBP1L* (*C10orf26*), top genes associated with the manic and depressive factors, respectively, have previously been implicated in SCZ (Schizophrenia Psychiatric GWAS Consortium, 2011;

see Table 1) though the former did not reach genome-wide significance with replication. *WBP1L* (*C10orf26*) is one of just four genes predicting *MIR137* target sites across three different prediction programs (TargetScan, PicTar, and miRanda; Lewis et al., 2005; Krek et al., 2005; John et al., 2004).

3.4 Gene pathway and gene ontology analyses

Using a conservative p-value cutoff (0.001) for gene pathway enrichment, analyses using ConsensusPathDB indicated that the MAN factor was enriched for 16 pathways (9 unique pathways with $q < 0.05$), including glutamatergic synapse, GABAergic synapse, neuronal system, and endocannabinoid signaling pathways. Gene pathway enrichment is presented in Table 2.

Similar analyses of the depressive factor identified enrichment of 16 gene pathways (7 unique pathways with $q < 0.05$) including developmental and axon guidance pathways. Finally, analyses of the POS factor identified 10 pathways (0 pathways at $q < 0.05$) again suggesting enrichment of developmental and axon guidance pathways.

Gene ontology analyses of the MAN factor successfully identified 86 categories with level 3 GO status at $p < 0.01$. Similar analyses of the DEP and POS phenotypes successfully identified 85 and 104 categories, respectively, with level 3 GO status at $p < 0.01$.

4. Discussion

In the present study, we meticulously derive quantitative phenotypes representing clinical features more specific to SCZ, and attempt to identify associated genetic variants by GWAS of each derived trait. Our results provide additional support for particular molecular pathways being relevant to SCZ etiology, further demonstrating that given sufficiently examined clinical heterogeneity, such pathways can be identified. When factor-analyzing symptom ratings in the SCZ-only case sample, affective factors emerged that more sensitively identify genes and pathways implicated in symptom presentation. Noting the potential of established genome-wide significance criteria to be overly conservative, we elected to correct for multiple testing using an FDR adjustment; this yielded a number of suggestive q-values. Pathways related to neurotransmission and vascular health demonstrated enrichment for trait effects.

Gene- and pathway-based analyses yielded results in support of the argument that symptom factors can more sensitively identify specific pathways relevant to schizophrenia. Deriving factor scores from SCZ-specific factor loadings may provide symptom phenotypes more sensitive in pathway enrichment analysis than do factors derived from a more heterogeneous sample. Results indicate that gene enrichment pathways affecting glutamate and GABA have an influence on affective symptom presentation in SCZ. There are multiple ways in which this could manifest, with one tentative example being an effect of GABA or glutamate on executive function and the prefrontal mechanisms underlying affective regulation. In addition, *PTPRG* and *WBP1L* have been previously associated with SCZ case status (SCZ GWAS Consortium, 2011; Ripke et al., 2013), but results from this study

suggest that the genes might act to modify affective symptoms in schizophrenia. These genes were not associated with case-control status in the ISHDSF.

Several gene-based analyses resulted in $q < .01$. These genes were consistent with neurobiological models of SCZ. Our results implicating GABA and glutamate gene pathways are consistent with the vast literature on GABA and glutamate in SCZ. GABA, the major inhibitory neurotransmitter in brain, plays an important role in normal brain functioning and GABA agonists are associated with improvement in core SCZ symptoms (for reviews of GABA in SCZ, see Wassef et al., 2003; Benes & Beretta, 2001; for GABA's relationship to the dopamine hypothesis, see Brisch et al., 2014).

The glutamate hypothesis of SCZ has been widely discussed (for a review, see Coyle, 2006) and it has been noted that drugs affecting glutamatergic transmission via NMDA receptor function have an influence on negative and cognitive symptoms of SCZ. GABAergic models are not inconsistent with glutamate-related NMDA receptor hypofunction, in that they focus on downregulation of parvalbumin expression and subsequent gamma dysfunction following administration of NMDAR antagonists (Lewis et al., 2012). Research investigating the clinical effectiveness of glutamate agonists for SCZ, Parkinson's, and obsessive-compulsive disorder (Javitt, 2012) suggests new avenues of pharmacotherapy for negative symptoms in addition to the D2 receptor antagonists currently used to manage psychotic symptoms. Alternatively, the clinical effectiveness of glutamate antagonists, such as ketamine for autism and treatment-resistant depression, has suggested a dynamic role for glutamate in brain across a wide array of psychiatric disorders (Javitt, 2011). Importantly, glutamate has been most recently implicated in schizophrenia in the largest genome-wide association study of schizophrenia to date (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

Overall, our enrichment findings implicating cGMP (triggering vasodilation) and cardiomyopathy are consistent with mounting evidence implicating vasodilation and vasculature in SCZ. Many of the replicated candidate genes for SCZ are expressed in vasculature (Schmidt-Kastner et al., 2006; 2012). Evidence also suggests that people with SCZ have greater vulnerability to cardiovascular disease (Hennekens et al., 2005) and that genetic pleiotropy exists between SCZ and risk for cardiovascular disease (Andreassen et al., 2013). People with SCZ appear to have distinct retinal vasculature (Meier et al., 2014), aberrant capillary dilation (Hudson et al., 1997; Ward et al., 1998; Lien et al., 2013), as well as atypical nailfold plexus vasodilation (Curtis et al., 1999; Vuchetich et al., 2008). The endothelial and mitochondrial dysfunction in SCZ have long been hypothesized to stem from vascular abnormalities (e.g., Cohen et al., 1995). Previous research has also identified atypical and simplified angioarchitecture and molecular alterations of the cerebral microvasculature in SCZ (Harris et al., 2008). In summary, previous research has provided ample evidence of vascular involvement in SCZ. Cardiomyopathy-related gene pathway enrichment found in this study is also consistent with previous case-control findings linking a cardiomyopathy-related gene (CMYA5, or myospryn) to risk for SCZ (Chen et al., 2011). These findings were first identified in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) sample and the Molecular Genetics of SCZ (MGS) sample, and were replicated in the Irish Family Sample.

Modest sample size is a limitation of this study, as is the large number of gene-based tests. However, with the sample size available the analyses resulted in several q values $< .01$ and several empirically supported gene pathways. It was also unexpected that manic and depressive factors should emerge foremost, given the number of studies citing negative symptom and disorganization factors. Nevertheless, the aim of this study was to remain agnostic with respect to symptom dimensions and to simply examine the genes associated with primary factors identified from the data.

Rich clinical data in symptom factor studies of schizophrenia genetics may serve as a compliment to large genome-wide efforts targeting risk loci and rare disruptive mutations (e.g., Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Purcell et al., 2014). Similar future analyses in a larger case sample might provide traction to these results, and could also help clarify the role of cardiovascular gene pathways in modulating affective symptoms in schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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(A)

(B)

Figure 1. Manhattan plots for variants with $p < 0.01$ for Manic (A), Depressive (B), and Positive (C) symptoms.

Table 1

Genes with gene-based analysis q values < 0.01 .

Gene	p -Value	q -Value	Chr.	Start	Length
<i>Manic Symptoms</i>					
RALYL	9.00e^{-08}	0.002	8	85095452	738627
IL4	2.51e^{-07}	0.006	5	132009677	8694
PTPRG ¹	3.74e^{-07}	0.009	3	61547242	733332
<i>Depressive Symptoms</i>					
TRAPPC9	2.51e^{-11}	$5.93\text{E}-07$	8	140742585	726094
CUX1	9.32e^{-09}	0.0002	7	101459183	468068
WBPI1 (C10orf26) ²	4.59e^{-08}	0.001	10	104503726	72296
<i>Positive Symptoms</i>					
TRAPPC13	9.36e^{-09}	0.0002	5	64920557	41398
PPWD1	7.36e^{-08}	0.002	5	64859065	24306
ZNF106	1.06e^{-07}	0.003	15	42704634	78762

Note: Chr. = chromosome, Start = start position, Length = number of base pairs.

¹ Stage 1 association with SCZ, no replication (SCZ GWAS Consortium, 2011).

² One of the 14 genes enriched for smaller p values in PGC data (Ripke et al., 2013).

Table 2Gene Pathway Enrichment ($q < 0.05$).

Factor	Pathway	Set Size	Candidates	q-Value	Source
MAN	GABA A receptor activation	12	5 (41.7%)	0.0364	Reactome
MAN	Cyclic GMP	18	6 (33.3%)	0.0364	Reactome
MAN	Dilated cardiomyopathy	90	16 (17.8%)	0.0159	KEGG
MAN	Hypertrophic cardiomyopathy	83	14 (16.9%)	0.0254	KEGG
MAN	Morphine addiction	93	15 (16.3%)	0.0254	KEGG
MAN	Glutamatergic synapse	116	18 (15.7%)	0.0159	KEGG
MAN	Retrograde endocannabinoid signaling	103	16 (15.7%)	0.0254	KEGG
MAN	Ion channel transport	158	22 (13.9%)	0.0159	Reactome
MAN	Transmembrane transport, small molecules	530	48 (9.1%)	0.0364	Reactome
DEP	Cyclic GMP	18	8 (44.4%)	0.0241	Reactome
DEP	Interaction between L1 and Ankyrins	26	10 (38.5%)	0.0167	Reactome
DEP	NRAGE signals cell death through JNK	45	13 (28.9%)	0.029	Reactome
DEP	Adherens junction	73	19 (26.0%)	0.00761	KEGG
DEP	L1CAM interactions	96	23 (24.0%)	0.00614	Reactome
DEP	Axon guidance	257	47 (18.3%)	0.00175	Reactome
DEP	Developmental Biology	373	63 (16.9%)	0.00123	Reactome

Note: MAN = Mania symptom factor, DEP = depression symptom factor. No POS symptom pathways met $q < 0.05$.