Journal of Genetics (2019) 98:60
https://doi.org/10.1007/s12041-019-1101-6

RESEARCH ARTICLE

Meta-analysis of genomic variants and gene expression data in schizophrenia suggests the potential need for adjunctive therapeutic interventions for neuropsychiatric disorders

S. ANIRUDH CHELLAPPA[1,2], ANKIT KUMAR PATHAK[3], PRASHANT SINHA[3], ASHWIN K. JAINARAYANAN[4], SANJEEV JAIN[2] and SAMIR K. BRAHMACHARI[1,3,5,6*]

[1] *Centre for Open Innovation – Indian Centre for Social Transformation (ICST), Bengaluru 560 001, India*
[2] *Department of Psychiatry, National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru 560 029, India*
[3] *Cluster Innovation Centre, University of Delhi, Delhi 110 007, India*
[4] *Indian Institute of Science, Education and Research, Mohali 140 306, India*
[5] *Council of Scientific and Industrial Research-Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi 110 025, India*
[6] *Academy of Scientific and Innovative Research (AcSIR), New Delhi 110 020, India*
*For correspondence. E-mail: skb@igib.in.

Received 30 September 2018; revised 22 January 2019; accepted 28 January 2019; published online 5 June 2019

Abstract. Schizophrenia (SZ) is a debilitating mental illness with a multigenic aetiology and significant heritability. Despite extensive genetic studies, the molecular aetiology has remained enigmatic. A recent systems biology study suggested a protein–protein interaction network for SZ with 504 novel interactions. The onset of psychiatric disorders is predominant during adolescence, often accompanied by subtle structural abnormalities in multiple regions of the brain. The availability of BrainSpan Atlas data allowed us to re-examine the genes present in the SZ interactome as a function of space and time. The availability of genomes of healthy centenarians and nonpsychiatric Exome Aggregation Consortium database allowed us to identify the variants of criticality. The expression of the SZ candidate genes responsible for cognition and disease onset was studied in different brain regions during particular developmental stages. A subset of novel interactors detected in the network was further validated using gene expression data of post-mortem brains of patients with psychiatric illness. We have narrowed down the list of drug targets proposed by the previous interactome study to 10 proteins. These proteins belonging to 81 biological pathways are targeted by 34 known Food and Drug Administration-approved drugs that have distinct potential for the treatment of neuropsychiatric disorders. We also report the possibility of targeting key genes belonging to celecoxib pharmacodynamics, Gα signalling and cGMP-PKG signalling pathways that are not known to be specific to SZ aetiology.

Keywords. schizophrenia; centenarians; interactome; BrainSpan; post-mortem; pathways; drug repurposing.

Introduction

Schizophrenia (SZ) is a complex psychiatric disorder with a multigenic aetiology, affecting almost 1% of the global population (McGrath *et al.* 2008). It has been clear that the disorder is highly heritable and there is a strong

SKB conceptualized and designed the project. ACS and AKJ performed the gene expression data analysis. AKP performed the meta-analysis of variants and PS constructed the spatio-temporal network. ACS and SKB wrote the manuscript. SJ provided intellectual support in interpreting the results and editing the manuscript.

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12041-019-1101-6) contains supplementary material, which is available to authorized users.

genetic basis, which has been a focus of research over the past decade (Cardno *et al.* 2012). Complex neuropsychiatric disorders like SZ, bipolar disorder (BP) and major depressive disorder (MDD) are driven by multiple genetic variants across various genomic loci that perhaps interact with environmental factors to produce the disease phenotype (Viswanath *et al.* 2018). The National Human Genome Research Institute of USA has catalogued 38 genomewide association studies (GWAS) (Hindorff *et al.* 2009), revealing the association of common variants with SZ (Girard *et al.* 2012). In addition, the Psychiatric Genomics Consortium (PGC) has identified 108 SZ-associated loci (Ripke *et al.* 2014). The molecular mechanisms by which these genetic variations contribute to psychoses could be better understood by studying protein–protein interactions (PPIs) and other molecular interaction networks. Recently, a novel random forest model named high-confidence protein–protein interaction prediction (HiPPIP) was developed to classify the pairwise features of interacting proteins (Ganapathiraju *et al.* 2016). The HiPPIP predicted 504 novel PPIs, adding to 1397 known PPIs, for 101 SZ candidate genes, presenting a novel theoretical interactome for SZ. A few (pairwise interactions) were experimentally validated (Ganapathiraju *et al.* 2016). The analysis illustrates that despite the divergent findings of different studies on SZ, a common thread emerges as the genes lead to pathways through the interaction network. Several genes present in key pathways deduced from the interactome are targets of existing drugs used to manage various chronic diseases.

While tissue-specific gene expression data from the Stanford Microarray Database (SMD) and tissue-specific gene expression and regulation database were included to build the HiPPIP model (Ganapathiraju *et al.* 2016), it still lacked a spatio-temporal information. SZ, a developmental disorder of largely adolescent onset is associated with subtle structural abnormalities and molecular differences in multiple brain regions (Howard *et al.* 2000; De Peri *et al.* 2012). Hence, there is a need to refine the network incorporating the available spatio-temporal data. While the HiPPIP has led to a large theoretically possible interactome, the biological networks *in vivo* are likely to be a subset of the computationally predicted network. This is mainly because the genes must be coexpressed and colocalized in order to interact. In addition, the biological relevance of the experimental evaluations carried out in noncentral nervous system tissues is debatable (Ganapathiraju *et al.* 2016). It would perhaps be more meaningful to evaluate the suspected targets in brains of patients with psychiatric illness.

Antipsychotics (APs) have been in use since the 1950s (Shen 1999). The first-generation APs were derived from a number of older drugs exploring antibiotic and anaesthetic effects, as well as drugs used in traditional medicine. At present, the commonly used drugs are second-generation APs, with their therapeutic effects largely being mediated by dopaminergic and serotonergic receptor blocking activities (Naheed and Green 2001). APs have been associated with long-term side effects such as weight gain (Sušilová *et al.* 2017), adverse metabolic effects, aggravating cognitive dysfunction (Zhang *et al.* 2017) and many others. Lithium and valproic acid have been administered to patients with BP but their mechanism of action is still not completely understood (Rogers and Taylor 2017). Thus, there is a pressing need for new drugs in psychiatry.

Therefore, by integrating genetic variation data from nonpsychiatric Exome Aggregation Consortium (ExAC) and centenarian genomes, along with gene expression data from BrainSpan Atlas and psychiatrically ill post-mortem brain samples, with the SZ interactome and gene–drug interaction network, we have performed a meta-analysis to improve the current understanding of the genomic and pharmacological complexity of neuropsychiatric disorders (Girard *et al.* 2012; Ripke *et al.* 2014; Farrell *et al.* 2015; Lanz *et al.* 2015; Ganapathiraju *et al.* 2016; Lek *et al.* 2016). The components of genomic variation associated with the disease are likely to influence the disease phenotype through changes in protein biology. The meta-analysis addresses the four mechanisms by which genomic variation could lead to the disease phenotype: by affecting the protein activity/function (identification of lethal nonsynonymous variations), quantity of protein (in normal and post-mortem brain tissues), timing (multiple developmental stages) and location (multiple brain regions) of protein production. In the absence of quantitative protein expression data, gene expression (mRNA abundance) is taken as a surrogate of the protein levels. The translational control and protein degradation pathways could not be a part of the analysis.

To begin with, the variants present in SZ genes were mined from Ensembl Variation (EV). The variants that were absent in genomes of healthy centenarians and nonpsychiatric ExAC database were identified and defined as variants of criticality. We harnessed the spatio-temporal gene expression data of SZ candidate genes from BrainSpan Atlas and integrated them into the existing SZ interactome to identify critical genes and interactors as potential targets for therapeutic interventions. We hypothesize that the resultant dynamic network and the interactome would be a better approximation of the real biological network of SZ genes in a developing human brain. We harnessed the transcriptome data of psychiatrically ill post-mortem brain tissues from Gene Expression Omnibus (GEO) (Lanz *et al.* 2015) to identify differentially expressed genes (DEGs) present in the SZ interactome. Some of the interactors provided insights into psychiatric disorders and associated comorbidities like inflammation, immune dysfunction and visual deficits. The druggable DEGs and their pathways were identified, presenting a probable subset of

targets for repurposing existing drugs for psychiatric disorders.

Materials and methods

The overall analysis is represented as a graphical abstract (figure 1a), while a detailed representation of the workflow is shown in figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/.

Database mining of single-nucleotide variants present in the candidate genes

A total of 123 genes (101 interactome candidate genes + 22 Online Mendelian Inheritance in Man (OMIM) genes) associated with SZ were retrieved from the literature (Ganapathiraju *et al.* 2016). The 101 interactome candidates were themselves derived from 77 GWAS (Ripke *et al.* 2014) and 25 historic/pre-GWAS genes (Farrell *et al.* 2015), with *GRM3* being common. Apart from this, the 22 OMIM genes associated with SZ were also retrieved from the supplementary material of Ganapathiraju *et al.* (2016). Their genomic co-ordinates (GRCh37) were extracted from Ensembl's Biomart (Yates *et al.* 2016). The gene symbols and their co-ordinates are shown in table 1 in electronic supplementary material. Nonsynonymous variants were mined from EV (Yates *et al.* 2016) (GRCh37) for the 123 candidate genes, of which 100 had well annotated nonsynonymous variations. The functional consequences of the variants were predicted using Polymorphism Phenotyping v2 (PolyPhen-2) (Adzhubei *et al.* 2010). The 'probably damaging' variants as predicted by PolyPhen-2 were queried in (i) genomes of healthy centenarians ($n = 93$) (Hariprakash *et al.* 2018) and (ii) nonpsychiatric ExAC database ($n = 47,082$) (Lek *et al.* 2016). Nonpsychiatric ExAC (v0.3) variants with genotype quality ≥ 20 and read depth ≥ 10 were used for the above analysis. The genes and variants were then screened against available literature and databases including OMIM (Amberger *et al.* 2015) to check for association with other chronic illnesses apart from SZ.

Construction of spatio-temporal dynamic network

The RNA-seq dataset from the BrainSpan Atlas of the developing human brain (Tebbenkamp *et al.* 2014) was retrieved for the SZ candidate genes using the R package *ABAEnrichment* (Grote *et al.* 2016), which contains expression data only for protein-coding genes (aligned to GRCh37). To increase the power in detecting developmental effects by using highly overlapping brain regions, the dataset for the enrichment analysis was restricted to the 16 brain regions sampled in five developmental stages. Among the 123 candidate genes, the spatio-temporal reads per kilobase of transcript per million mapped reads (RPKM) values were available only for 96 (89 interactome candidates + seven OMIM candidates) genes and the remaining 27 genes and their interacting partners, if any, were excluded from the analysis. The raw data were z-score normalized (genewise) and the median absolute deviation (MAD) (scaling factor, $k = 1$) of the expression was calculated for each gene across all 16 tissues in five developmental stages. To facilitate the understanding of PPI network dynamics with respect to the brain regions and developmental stages, we developed an open source network visualization toolkit. The toolkit is written in JavaScript using ReactJS [https://reactjs.org/], SigmaJS (http://sigmajs.org) and D3 (https://d3js.org) packages. This toolkit is accessible publicly at placet (https://placet.noop.pw/) [use updated version of Google Chrome for best results] and the source code can be accessed from https://github.com/prashnts/placet. Hyperlink-induced topic search (HITS) was used to rank the genes in the network based on the degree of the nodes. The normalized spatio-temporal gene expression data were integrated into the interactome, with the node sizes representing the expression levels of the corresponding genes. To classify the druggable genes from the remaining causative candidates, we employed hierarchical classification of spatio-temporal expression data of 96 SZ genes. This spatio-temporal dynamic network is accessible publicly at placet (https://placet.noop.pw/) and the Source code can be accessed from https://github.com/prashnts/placet.

Post-mortem microarray data analysis

Microarray expression profiles of 54,675 Affymetrix probe sets of pre-frontal cortex (PFC), hippocampus (HPC) and striatum (STR) from 205 subjects including those diagnosed with SZ, BP and MDD, along with clinically matched healthy controls were downloaded from GEO (ID: GSE53987) (Lanz *et al.* 2015). The downloaded data were MAS 5.0 normalized and $\log_2$ transformed to make sure that the data followed a Gaussian distribution. The distribution was looked up for samples that might show variations in gene expression. The mean of expression of each gene across its corresponding samples was calculated. The fold change (FC) was calculated between the gene expression means of cases and corresponding controls. Student's t-test was used to test for differences in gene expression between cases and controls. The false discovery rate (FDR) of t-test P values was calculated for multiple hypothesis testing using the Benjamini–Hochberg (BH) method (table 2 in electronic supplementary material). A two-fold change (FC > 2) in gene expression in cases compared to controls along with a $P < 0.01$ was considered to be differentially expressed. Annotation of Affymetrix probe IDs was performed using

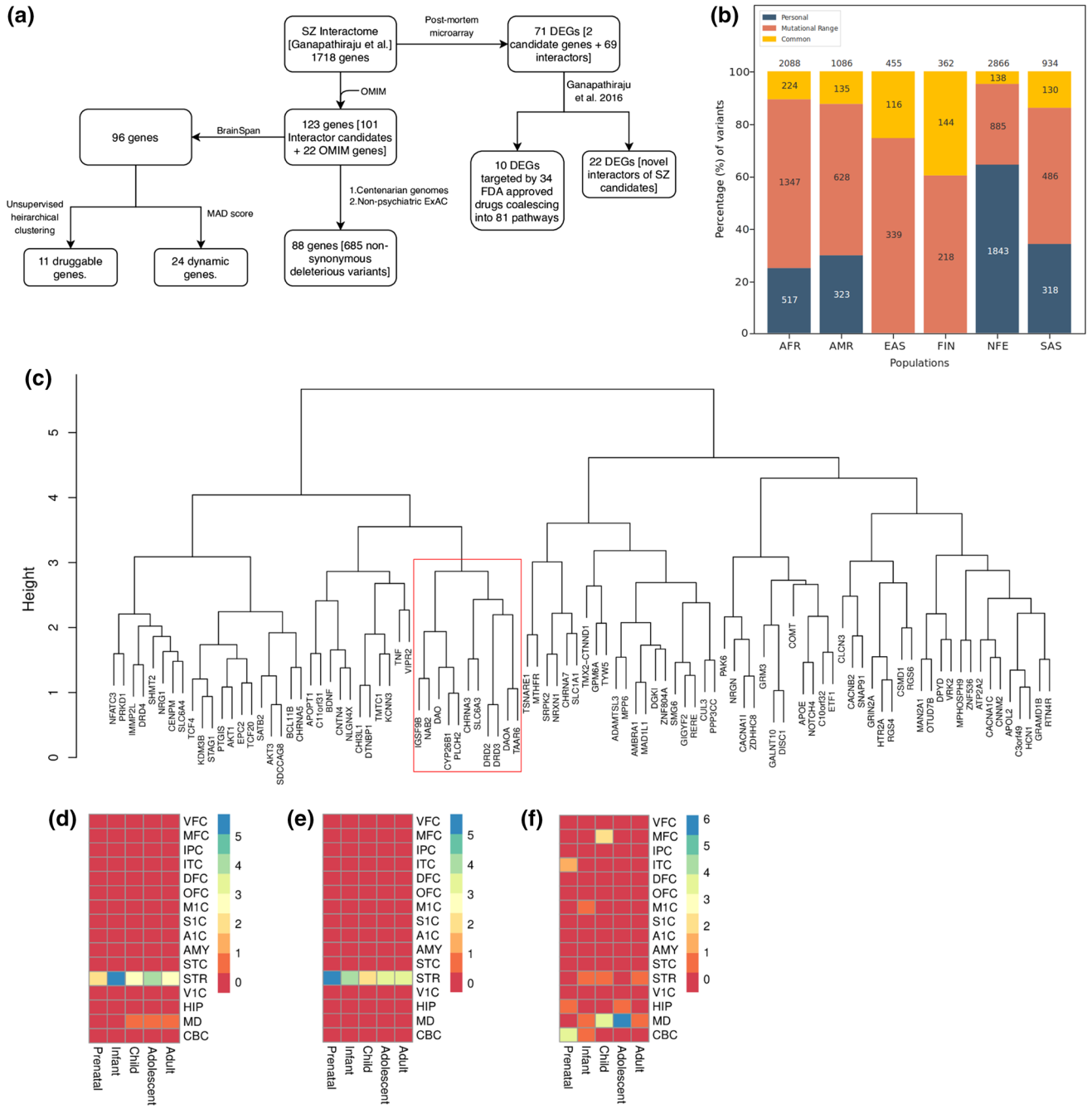

Figure 1. (a) Workflow: meta-analysis of genomic variants and gene expression to identify repurposable drugs for SZ. (b) Population distribution of personal, mutational range and common nonsynonymous variants in SZ genes from the nonpsychiatric ExAC database. Amongst 4495 variants, 4045 mapping to 99 (out of 100) SZ genes were identified in six populations. The variants observed in each population are directly proportional to their sample size. The bar diagram represents personal variants in blue colour, the mutational range variants in red colour and the common variants in yellow colour. AFR: African/African American; AMR: Latino; EAS: East Asian; FIN: Finnish; NFE: Non-Finnish European and SAS: South Asians. (c) 11 druggable genes classified based on similarity in gene expression. (d–f) spatio-temporal expression profiles (scale: z-score [RPKM]) of druggable SZ candidates: (d) DRD2, (e) DRD3 and (f) SLC6A3 in a developing human brain.

Affymetrix Netaffx Batch Query (http://www.affymetrix.com/analysis/index.affx). The union set of all DEGs in nine different cases was identified. Ganapathiraju *et al.* (2016) had identified 504 novel PPI in addition to the 1397 PPI, comprising 1901 interactions in total. We have arrived at 1718 genes by identifying the union set of all genes present in the 1901 interactions. We then overlapped the union set of all DEGs identified, with all 1718 genes in the SZ interactome, for the downstream analysis.

Identification of druggable genes and pathways

A 2-D matrix representing 286 biological pathways involving 122 druggable genes was constructed from the literature (Ganapathiraju *et al.* 2016). The DEGs identified from the post-mortem brain tissues were overlapped with 122 druggable genes to identify the drug targets in the interactome that are differentially expressed from the post-mortem microarray study. An independent analysis was carried out using ConsensusPathDB (Release 32) (Kamburov *et al.* 2011) to identify more druggable genes (apart from 122) in biological pathways to which the SZ candidate genes have been attributed ($P < 0.01$).

Statistical analysis and data visualization

All the statistical tests and data visualization were performed using *R*, including the MAS-5.0 normalization and statistical corrections of microarray gene expression data.

Results

Functional consequences of nonsynonymous variants

To characterize the functional implications of the nonsynonymous variants in SZ candidate genes, we mined data from EV. Of the 123 SZ candidate genes (101 interactome candidate genes +22 OMIM genes), EV reported 4495 well annotated nonsynonymous variants in 100 SZ candidate genes for which the PolyPhen scores were retrieved (table 3a in electronic supplementary material).

***Identification and shortlisting of lethal variants using genomes of healthy centenarians and nonpsychiatric ExAC database*:** According to PolyPhen analysis, it was observed that 2037 (of 4495) variants were called as probably damaging, which mapped to 99 (of 100) SZ genes. To narrow down the number of deleterious variants, we eliminated 33 variants of the 2037 variants, belonging to 24 genes that were observed in genomes of healthy centenarians ($n = 93$) (table 3b in electronic supplementary material). Of the 2004 variants absent in centenarians (table 3b in electronic supplementary material), (i) we found that 265 variants (mapping to 79 genes) were also absent in nonpsychiatric ExAC database and were defined as variants of criticality (table 3b in electronic supplementary material) and (ii) we retained the remaining 1739 lethal variants, i.e. those absent in centenarians but present in nonpsychiatric ExAC, that mapped to 99 genes that could turn deleterious later on under certain circumstances (table 3b in electronic supplementary material). These 1739 variants were further classified into three categories based on their allele frequencies (AFs) (AF < 0.0001: personal; AF: 0.0001 to 0.01: mutational range; AF > 0.01: common). Among the 1739 lethal variants, 1319 (mapping to 98 genes) were personal, 405 (mapping to 75 genes) were in the mutational range and only 15 (mapping to 10 genes) were common in populations. We limited our analysis to common and mutational range variants but not the personal variants since the association of individual personal variants in complex disorders like SZ may represent a very small proportion of the possible risk factors, and unlikely to contribute to susceptibility, at the population level. Thus, the 265 variants of criticality might act alone, or in combinations with the 15 common and 405 mutational range variants, i.e. 685 variants in total, mapping to 88 SZ genes (79 high risk genes + nine genes unique to the mutational range and common variant genes), to contribute to the disease phenotype (figure 1a; figure 1 in electronic supplementary material). Hence, we present a panel of potentially deleterious 685 variants that could be further investigated for behavioural phenotypes and brain pathobiology in animal models of neuropsychiatric disorders (table 3b in electronic supplementary material). It was also witnessed that six (*CSMD1*, *CACNA1C*, *PLCH2*, *NRG1*, *ADAMTSL3* and *TCF20*) of 88 SZ candidate genes had a relatively higher burden of nonsynonymous variants (> 20 variants per gene) (figure 2 in electronic supplementary material). It is interesting to note that although the number of variants reduced at every step during the variant filtration process, the number of genes remain fairly the same. This could be because the risk variants are distributed among genes identified by the GWAS and other association studies but seldom cluster onto a particular locus.

***Distribution of nonsynonymous variants present in SZ genes in global populations*:** To gain an overall snapshot of the AFs of the variants present in SZ genes in global populations, we queried all the original 4495 variants in a nonpsychiatric ExAC database. Based on analysis, it was observed that 4045 variants were mapped to 99 genes, thereby discarding the 450 variants that were absent in the nonpsychiatric ExAC database. The AFs of 4045 variants in six populations reported in the nonpsychiatric ExAC database were also retrieved (table 3c in electronic supplementary material). The analysis revealed that the number of variants observed in each of the populations was directly proportional to their sample size. However, the proportion of the personal variants was higher ($n = 1843$) in the outbred European population (NFE) but was absent in the inbred Finnish (FIN) and East-Asian Tibeto-Burman (EAS) population (figure 1b). Although the personal variants were absent in FIN and EAS, the prevalence of psychiatric disorders was found to be as high in both the populations (Lehtinen *et al.* 1990). Thus, the absence of personal variants in the FIN and EAS populations could be an artefact of the under-representation of the corresponding cohorts in the ExAC database.

***Literature mining of variants present in SZ candidate genes that have been associated with multiple chronic illnesses*:** To verify the association of SZ candidate genes with other chronic illnesses, we utilized OMIM, literature in PubMed and other online sources, which revealed 94 disease-associated nonsynonymous variants present in 37 SZ genes, i.e. almost 40% of all SZ candidates (Amberger *et al.* 2015). These variants were associated with other disorders including BP, MDD, autism, epilepsy, seizures, Alzheimer's, diabetes, hypertension etc. (table 3d in electronic supplementary material). Among the 94 variants, only 22 were predicted to be lethal by PolyPhen analysis which highlights the ambiguities inherent in the current methods in predicting the protein deleteriousness. Of these 22 presumed lethal variants, 10 (rs769455, rs1801158, rs34845648, rs45571736, rs2904552, rs3970559, rs2229961, rs34622148, rs1801500 and rs8192466) were found to be absent in centenarian but present in the nonpsychiatric ExAC database. However, none of the 22 was absent both in the centenarians and the nonpsychiatric ExAC database.

Analysis of the spatio-temporal interactome

Although the PPI map for SZ presented all the possible interactions, a large proportion of the genes represented in the interactome are not coexpressed in a given location of the brain at a particular developmental stage. Therefore, we retrieved and integrated the spatio-temporal gene expression data from BrainSpan Atlas into the existing SZ interactome, thereby redefining the network as a function of space (16 brain regions) and time (five developmental stages) (placet) (table 4 and figure 3 in electronic supplementary material).

***Extent of difference in gene expression between hub genes and nonhub genes*:** HITS was used to rank the genes as hubs (top 10 genes) or nonhubs (bottom 10 genes). From this study, we hypothesized that highly connected genes, i.e. the hub genes, must be expressed significantly higher compared to nonhub genes in order to interact with a larger set of proteins. However, no difference was found between the gene expression means of hub genes and nonhub genes.

***Characterization of gene expression dynamics in regions of adolescent and adult brain*:** To identify the genes that exhibit high variations in the expression pattern in a normal human brain, we carried out MAD score analysis for the 96 SZ candidate genes across the spatio-temporal gene expression data. The analysis revealed that the expression of 24 genes was highly dynamic across space and time among which 13 (*RGS4*, *HTR2A*, *APOL2*, *GRIN2A*, *CNNM2*, *CACNA1C*, *ZDHHC8*, *HCN1*, *DPYD*, *OTUD7B*, *ZNF536*, *C3orf49* and *CLCN3*) were highly expressed in adult and/or adolescent brain tissues compared to child, infant and prenatal brain tissues (table 5 in electronic supplementary material).

***Expression based similarity search for druggable SZ candidate genes*:** Despite the large GWAS findings, it is still not clear, which, if any, of the newly identified GWAS loci will serve as good starting points for drug development in SZ (Dolgin 2014). Most drug targets may not be ubiquitously expressed but enriched and localized in distinct tissues relevant to the disorders, even under normal conditions (Kumar *et al.* 2016). Hence, there is a need to classify the drug targets from candidate genes based on their expression patterns. To classify the druggable genes from the remaining causative candidates, we employed hierarchical classification of spatio-temporal expression data of 96 SZ (the file that contains 'Druggability signatures of 11 SZ candidate genes...' in electronic supplementary material) candidate genes, and identified a subcluster of 11 genes (*IGSF9B*, *NAB2*, *DAO*, *CYP26B1*, *PLCH2*, *CHRNA3*, *SLC6A3*, *DRD2*, *DRD3*, *DAOA* and *TAAR6*) (figure 1c) which were enriched only in certain brain regions at certain developmental stages. Amongst the sub-cluster of 11 genes, three (*DRD2*, *DRD3* and *SLC6A3*) have been modestly implicated in the action of anti-psychotic drugs (figure 1, d–f). The druggability signatures of all the 11 genes are discussed in the file that contains 'Druggability signatures of 11 SZ candidate genes...' in electronic supplementary material.

***Analysis of DEGs in psychiatrically ill post-mortem brain tissues and their overlap with the SZ interactome*:** To identify the genes present in the SZ interactome that are dysregulated in psychiatric patients, the microarray expression profiles from 205 post-mortem brains of patients and matched controls were examined (Lanz *et al.* 2015). Analysis of expression profiles of PFC, HPC and STR revealed 985 unique DEGs (FC $>$ 2; $P < 0.01$) under nine different conditions (table 2 and figure 4 in electronic supplementary material). The raw *t*-test *P* values were used since FDR (BH) corrected *P* values were not significant for most genes to be called as differentially expressed. To validate the role of genes from the SZ interactome in post-mortem brain tissues, we overlapped the gene IDs of 985 DEGs and 1718 genes in the SZ interactome. We obtained an overlap of 71 genes (two candidate genes +69 interactors) that were present in the SZ interactome and also differentially expressed in post-mortem brain tissues of which 22 were novel interactors, as predicted by Ganapathiraju *et al.* (2016). Fourteen of the 22 dysregulated genes in our analysis revealed direct or indirect relationship with neuropsychiatric disorders and comorbidities from previous studies (table 1). The remaining eight novel interactors (*MYOZ2*, *CARS*, *GSC2*, *MKI67*, *ZC3H15*, *HOPX*, *CDC42S1E* and *VANGL1*) though dysregulated in our analysis had no previous mention in the literature

with psychoses. Based on the analysis, it was observed that only two SZ candidate genes (*SLC6A4* and *CACNB2*) were differentially expressed in HPC (BP) ($\log_2$FC = 1.31; P = 0.005) and PFC (MDD) ($\log_2$FC = −1.11; P = 0.008), respectively (table 2 in electronic supplementary material). Interestingly, 14 of 71 DEGs were previously identified as druggable targets of various food and drug administration (FDA)-approved drugs in the gene–drug interactome study by Ganapathiraju *et al.* (2016). The above analysis is illustrated in figure 4 in electronic supplementary material.

Analysis of druggable genes and pathways

A 2-D matrix representing 286 biological pathways involving 122 druggable genes was reconstructed from the literature. From the above post-mortem gene expression data of the patients, we identified 14 druggable DEGs of which four (*PTGS1*, *ERBB2*, *PTGER3* and *ESR2*) were found to be downregulated in at least one of the nine conditions, as mentioned in the previous section. Since inhibition of highly expressed genes would be easier (*It's all druggable* 2017 *Nat. Genet.* **49**, 169–169, is an editorial review by *Nature Genetics*), the four downregulated targets were excluded from downstream analysis. This resulted in 10 upregulated and probable drug targets including *ACE*, *CD44*, *mTOR*, *RARA*, *PTPN1*, *LDLR*, *CD3E*, *NOS3*, *CFTR* and *CASR*, targeted by 34 FDA-approved drugs (table 2) (subdivisions A&B in electronic supplementary material) belonging to 81 biological pathways (table 6 in electronic supplementary material).

***Investigation into druggable genes present in pathways belonging to the SZ candidate genes*:** Among the 71 DEGs identified from post-mortem brains, only two were candidate genes (*SLC6A4* and *CACNB2*) and 10 were druggable interactors. To identify more druggable gene upstream or downstream in the biological pathways associated with SZ, we used ConsensusPathDB (CPDB) to identify pathways to which the original list of 123 SZ candidate genes belong (P < 0.01). The CPDB analysis revealed over-representation of 46 (of 123) genes in 54 biological pathways which includes dopaminergic signalling, MAPK signalling, cAMP signalling, axon guidance, calcium signalling, G α signalling, celecoxib pharmacodynamics, T-cell receptor signalling, cGMP-PKG signalling, Alzheimer's disease pathway etc. (table 7 in electronic supplementary material). Of these 54, only three pathways (celecoxib pharmacodynamics, G α signalling and cGMP-PKG signalling) had putative four drug targets (COX2, PLCB1, GNAQ and PDE10A), which we suggest to be further investigated for drug repurposing. The pathways, drug targets and FDA-approved drugs targeting them have been described in subdivisions A&B in electronic supplementary material, while their expression profiles are presented in figures 5–8 in electronic supplementary material.

Discussion

Despite extensive genetic studies of neuropsychiatric disorders, the molecular mechanisms of patho-biology are still unknown. A computational systems biology study had identified protein interactions of SZ candidate genes and predicted a large number of novel interactions and interactors among which several were targets of FDA-approved drugs (Ganapathiraju *et al.* 2016). With the availability of the genomes of healthy centenarians, nonpsychiatric ExAC and BrainSpan data, we have made an attempt to identify relevant candidate genes in the network that could influence the risk of psychoses. By leveraging centenarian genomes and nonpsychiatric ExAC data, the risk was narrowed down to 685 variants, spread over 88 SZ candidate genes that could be investigated further using animal models. Literature mining suggested that ∼40% of all SZ GWAS genes have shared genetic risk for one or more chronic illnesses, which needs to be validated by meta-analysis of genetic and clinical phenotype data.

BrainSpan data suggested 13 dynamic and highly expressed genes in adult and adolescent brain regions, which might play a crucial role in the onset of psychiatric illnesses. Expression-based similarity search of druggability in normal human brain suggested the prioritization of 11 SZ candidate genes that could be potential targets of novel or repurposed drugs. It has been evident in recent years that a significant number of genes and molecular pathways are commonly dysregulated across a spectrum of psychiatric disorders including SZ, BP and MDD (Brainstorm Consortium *et al.* 2018; Gandal *et al.* 2018; Ruderfer *et al.* 2018). Thereby, we felt that observing differential expression across a spectrum of psychiatric disorders might be a better way to look for druggable genes in the interactome. A total of 22 novel interactors present in the SZ interactome were found to be dysregulated in post-mortem brains who were diagnosed for various psychiatric disorders. These proteins previously had null or minimal associations with psychoses, thereby now validating a subset of the novel interactors as predicted by Ganapathiraju *et al.* (2016). We also observe the dysregulation of *DHDDS*, a gene that has been strongly associated with retinitis pigmentosa, which occasionally co-occurs in certain SZ cases (table 1) (McDonald *et al.* 1998). Although no direct evidence for psychoses was found for eight novel interactors that were dysregulated in psychiatric post-mortem brains, some of them (*MYOZ2*, *GSC2*, *MKI67* and *VANGL1*) were discernible and need further investigation as they point to critical processes. *MYOZ2* belongs to a family of sarcomeric proteins that bind to calcineurin, a phosphatase involved in calcium and calcineurin signalling, which are critical for SZ biology (Lidow 2003;

Table 1. Dysregulated novel interactors identified from post-mortem microarray analysis.

Gene ID	Disorder (tissue)	$\log_2$FC	*P* value	Previous evidence of association	References
PCDHGC5	SZ (PFC)	−1.09	0.004	Differentially expressed in differentiated neurons from clozapine responders	Nakazawa *et al.* (2017)
PDGFB	SZ (PFC)	1.16	0.001	*De novo* missense mutation associated with BD	Kataoka *et al.* (2016)
	MDD (PFC)	1.08	0.004		
GNAS	SZ (HPC)	−1.65	0.003	Differentially methylated region in monozygotic twins discordant for SZ	Castellani *et al.* (2015)
	MDD (HPC)	−1.17	0.004		
CD3E	SZ (HPC)	1.28	0.003	Associated with immunodeficiency	Soudais *et al.* (1993)
	BP (HPC)	1.11	0.005	Polymorphisms associated with antidepressant response in Mexican–Americans with MDD	Wong *et al.* (2008)
DHDDS	BP (HPC)	−1.14	0.001	Missense mutations associated with retinitis pigmentosa (RP) in Ashkenazi Jews; RP and SZ co-occur in some patients	McDonald *et al.* (1998), Zelinger *et al.* (2011), Narayanaswamy *et al.* (2013)
CD44	SZ (STR)	1.03	0.006	Upregulated in SZ post-mortem DFC	Fillman *et al.* (2013)
ATP6V0A2	BP (PFC)	1.25	0.005	Mutations in the same region repeatedly linked with BP	Serretti and Mandelli (2008)
ERAP2	BP (PFC)	−1.09	0.004	A functional variant (rs3813065/-442 C/T) on the *PIK3C3* gene which regulated the expression of *ERAP2* was associated with increased risk to SZ in Chinese individuals.	Tang *et al.* (2008), Kariuki *et al.* (2010)
CD9	BP (PFC)	−1.19	0.004	Dysregulation observed along with myelination-related genes	McCullumsmith *et al.* (2007)
APOL1	BP (STR)	1.11	0.0003	SNPs found in strong haplotype in SZ affected families	Takahashi *et al.* (2008)
CASR	BP (STR)	1.04	0.005	Upregulated in ischemic brain injury	Kim *et al.* (2014)
AGF1G1	BP (STR)	1.11	0.008	Upregulated in SZ lymphoblastoid cell lines	Sanders *et al.* (2017)
PPP1R11	MDD (HPC)	−1.36	0.008	Resides within MHC class 1 loci, SZ hotspot	Mokhtari and Lachman (2016)
TACR3	MDD (HPC)	−1.61	0.001	Insignificant association for genotype/haplotype markers in Japanese populations	Saito *et al.* (2008)

Table 2. Shortlisted druggable genes and their corresponding FDA-approved molecules.

Drug target (gene symbol) ($n = 10$)	Gene name	Interacting drugs (FDA approved)	No. of FDA-approved drugs ($n = 34$)	Relevance of the gene to psychoses or neurobiology	Support for druggability (animal model etc.)
ACE	Angiotensin converting enzyme	Ramipril, fosinopril, trandolapril, benazepril, enalapril, moexipril, perindopril, quinapril, rescinnamine, captopril, cilazapril, spirapril and temocapril	13	Eckman *et al.* (2006), Gadelha *et al.* (2015a, b), Nadalin *et al.* (2017)	AbdAlla *et al.* (2013), Hobgood (2013), Gadelha *et al.* (2015b)
CD44	Cell-surface glycoprotein	Hyaluronan	1	Ponta *et al.* (2003)	NA
mTOR	Serine/threonine-protein kinase	Everolimus, temsirolimus, sirolimus and pimecrolimus	4	Kim *et al.* (2002), Pham *et al.* (2016), Wang *et al.* (2017)	Tufts SZ mTOR studies; Zhou *et al.* (2013), Lipton and Sahin (2014)
RARA	Retinoic acid receptor alpha	Acitretin, adapalene, tazarotene, alitretinoin and etretinate	5	Haybaeck *et al.* (2015), Lerner *et al.* (2016)	Rioux and Arnold (2005), Malaspina and Michael-Titus (2008), Jarvis *et al.* (2010), Haybaeck *et al.* (2015)
PTPN1	Tyrosine-protein phosphatase non-receptor type 1	Tiludronate	1	Freedman *et al.* (2001), Imming *et al.* (2006), Yin *et al.* (2017)	Carty *et al.* (2012), He *et al.* (2014)
LDLR	Low-density lipoprotein receptor	Porfimer	1	Nourooz-Zadeh *et al.* (1996), Gibbons *et al.* (2010)	Gibbons *et al.* (2010)
NOS3	Nitric oxide synthase 3	Miconazole, tetrahydrobiopterin and L-arginine	3	Marsden *et al.* (1993), Shinkai *et al.* (2002), Pilar (2008)	Wass *et al.* (2008), Lafioniatis *et al.* (2016), Pitsikas (2016)
CFTR	Cystic fibrosis transmembrane conductance regulator	Glyburide, ivacaftor, ibuprofen and bumetanide	4	Gillen and Harris (2012)	Puljak and Kilic (2006)
CASR	Calcium-sensing receptor	Cinacalcet	1	Gupta *et al.* (2007), Hendy *et al.* (2013)	Kim *et al.* (2014), Dal *et al.* (2015)
CD3E	T-cell surface glycoprotein CD3 epsilon chain	Muromonab	1	NA	Hoosain *et al.* (2015)

Miyakawa *et al.* 2003). *GSC2*, a homeodomain containing gene that residing on 22q11, which is a known hotspot for psychoses (Saleem *et al.* 2001). *MKI67* encodes a nuclear protein that is associated with cellular proliferation and it has often been suggested that SZ is a disorder of inappropriate neuronal proliferation and pruning (Keshavan *et al.* 1994). Mutations in *VANGL1* are associated with neural-tube defects (Kibar *et al.* 2009) which have also been associated with increased risk in SZ patients (Zammit *et al.* 2007).

Of the 10 druggable interactors that are shortlisted for repurposing (table 2), it would be meaningful to investigate the action of drugs in the context of receptor-based (*CD44*, *RARA*, *LDLR*, *CASR* and *CD3E*) and nonreceptor targets (*ACE*, *mTOR*, *PTPN1*, *NOS3* and *CFTR*) in ameliorating the whole spectrum of psychiatric symptoms. The druggable genes that were further identified in pathways involving the SZ candidates, including *COX2*, *PLCB1*, *GNAQ* and *PDE10A*, were found to be highly expressed in the developmental stages that are pertinent to the onset of psychiatric illness. Thus, investments must be made into experimental validation in confirming the role of the above four genes and interacting small molecules in ameliorating SZ-like symptoms in animal models.

Our work essentially builds on the findings of Ganapathiraju *et al.* (2016), which itself were derived based on Ripke *et al.* (2014) and Farrell *et al.* (2015). Hence, we have also made an independent attempt to compare our results with the findings of a recent GWAS (Ruderfer *et al.* 2018) that had identified 114 genomewide significant loci (mapping to 57 genes) associated with SZ and BP, of which 22 genes overlap with our original set of 123 genes while the remaining 35 are unique to Ruderfer *et al.* (2018) (table 8 in electronic supplementary material). The spatio-temporal expression profiles were available in BrainSpan for only 15 of the 35 genes (figure 9 in electronic supplementary material). Two of these, *KCTD13* and *STK4*, are interactors of SZ candidate genes *CUL3* and *AKT1*, respectively. However, none of the 15 was found to be differentially expressed in the post-mortem microarray data. Analysing results from the genetic association studies from the disorders as complex as SZ are complicated. Sometimes the variants discovered in genes may be at stronger eQTLs but not as significantly associated. This makes a causal interpretation much more difficult. The biological pathways, though diverse, cover a broad spectrum of cellular functions such as viability, proliferation and regulation of cell motility which are generic but may be critical to the pathobiology of SZ. It is now fairly evident that the drugs that rely predominantly on modifying dopaminergic or serotonergic neurotransmission may be inadequate to address the complexity of the biological processes that we are now beginning to understand. One size, indeed, may not fit all. Hence, there is a pressing need for adjunctive therapeutic strategies targeting the genes and pathways that are being detected by current research. Validation of these proposed drugs, drug targets and pathways in animal models and induced pluripotent stem cell-derived neuronal lineages of SZ patients (Viswanath *et al.* 2018) could be useful to help unravel the biology of mental illness and also accelerate the drug repurposing pipelines.

Acknowledgements

SKB is a recipient of the J. C. Bose National Fellowship. ACS thanks Mohandas Pai foundation for providing fellowship support through Centre for Open Innovation, IndianCST. We thank Raja Seevan, Sri Kumar and the IndianCST team for the infrastructure support. We thank NIMHANS for providing institutional support to SJ. We thank N. Balakrishnan for providing access to the computational facility at the Supercomputer Education and Research Centre, Indian Institute of Science. We also thank Vinod Scaria for providing access to the allele frequencies from his centenarian genome data and Beena Pillai for inputs on gene expression data analysis. We finally thank Meera Purushottam, Ramakrishnan Kannan, Biju Viswanath and Ravi Kumar Nadella for critical reading of this manuscript.

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Corresponding editor: S. Ganesh