Multi-scale analysis of schizophrenia risk loci: Integrating centenarian genomes and spatio-temporal expression profiles suggests the need for adjunctive therapeutic interventions for neuropsychiatric disorders.

Anirudh Chellappa S\(^1\),4, Ankit Kumar Pathak\(^2\), Prashant Sinha\(^2\), Ashwin K. Jainarayanan\(^3\), Sanjeev Jain\(^4\), Samir K. Brahmachari\(^{1,2,5,6,*}\)

\(^1\)Centre for Open Innovation - Indian Centre for Social Transformation, Bengaluru, India

\(^2\)Cluster Innovation Centre, University of Delhi, Delhi, India

\(^3\)Indian Institute of Science, Education and Research, Mohali, India

\(^4\)Department of Psychiatry, National Institute of Mental Health and Neurosciences, Bengaluru, India

\(^5\)CSIR – Institute of Genomics and Integrative Biology, New Delhi, India

\(^6\)Academy of Scientific and Industrial Research, New Delhi, India

Running title: Multi-scale analysis of schizophrenia risk loci

* To whom correspondence should be addressed: Prof. Samir K Brahmachari, CSIR – Institute of Genomics and Integrative Biology, New Delhi, India 110007,

Tel.: (91) 9810443272; Email: skb@igib.in
ABSTRACT

Schizophrenia (SZ) is a debilitating mental illness with multigenic etiology and significant heritability. Despite extensive genetic studies the molecular etiology has remained enigmatic. A recent systems biology study suggested a protein-protein interaction (PPI) network for SZ with 504 novel interactions. The onset of psychiatric disorders is predominantly 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 SZ interactome as a function of space and time. The availability of genomes of healthy centenarians and non-psychiatric ExAC database allowed us to identify the variants of criticality. The expression of SZ candidate genes responsible for cognition and disease onset were 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 FDA approved drugs that have distinct potential for treatment of neuropsychiatric disorders. We also report the possibility of targeting key genes belonging to Celecoxib pharmacodynamics, Gα signaling and cGMP-PKG signaling pathways, that are non-specific to schizophrenia etiology.

Keywords: Schizophrenia, centenarians, interactome, BrainSpan, post-mortem, pathways, drug repurposing.

INTRODUCTION

Schizophrenia (SZ) is a complex psychiatric disorder with multi-genic aetiology, affecting almost 1% of the global population (McGrath J et al., 2008). It has been clear that the disorder is highly heritable and there is a strong genetic basis, which has been a focus of research over the past decade (Cardno et al., 2012). Complex neuropsychiatric disorders like SZ, Major depressive disorder (MDD), Bipolar disorder (BP), Obsessive compulsive disorder (OCD) and Autism spectrum disorder (ASD) 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 (NHGRI) of USA has catalogued 38 genome-wide association studies (GWAS) on SZ (Hindroff 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 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. 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 (pair-wise 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 (Ganapathiraju et al., 2016).

While tissue specific gene expression data from the Stanford Microarray Database (SMD) and Tissue-specific Gene Expression and Regulation (TiGER) database were included to build the HiPPIP model, 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 co-expressed and co-localized in-order to interact. In addition, the biological relavance of the experimental evaluations carried out in non-CNS 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 (AP) have been in use since 1950s (Shen et al., 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 et al., 2001). Antipsychotics have been associated with long-term side effects such as weight gain (Susilova 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 bipolar disorder, but their mechanism of action is still incompletely understood (Rogers et al., 2017). There is thus a pressing need for new drugs in psychiatry.

Hence, by integrating data points from non-psychiatric ExAC, centenarian genomes, Allen Brain Atlas, expression profiles of psychiatrically ill post-mortem brain samples, SZ interactome and gene-drug interaction network, we present a multi-scale analysis to improve the current understanding of the genomic and pharmacological complexity of neuropsychiatric disorders (Girard et al., 2012; Ripke et al., 2014; Farrell MS et al., 2015; Ganapathiraju et al., 2016; Exome Aggregation Consortium et al., 2016; Lanz et al., 2015). The components of genomic variation associated with the disease, are likely to influence the disease phenotype through changes in protein biology. Our “multi-scale” analysis addresses the 4 mechanisms by which genomic variation could lead to the disease phenotype; by affecting the protein activity/function (identification of lethal non-synonymous 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. The variants that were absent in genomes of healthy centenarians and non-psychiatric ExAC database was 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 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 analysis is represented as a graphical abstract (Figure 1)

Database mining of single nucleotide variants present in the candidate genes:
123 genes (101 interactome candidate genes + 22 OMIM genes) associated with SZ were retrieved from literature (Ganapthiraju et al., 2016). The 101 interactome candidates were themselves derived from 77 GWAS (Ripke et al., 2014) and 25 historic/pre-GWAS genes (Farrell MS et al., 2015), with GRM3 being common. Apart from which, the 22 OMIM genes associated with SZ were also retrieved from the supplementary material of Ganapthiraju 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 given in Supplementary File 1. Well annotated non-synonymous variants were mined from Ensembl Variation (Yates et al., 2016) (GRCh37) for 123 candidate genes, of which 100 had well annotated non-synonymous 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 were queried in (i) genomes of healthy centenarians (N=93) (http://clingen.igib.res.in/sage/) and (ii) non-psychiatric ExAC database (N=47,082) (Exome Aggregation Consortium et al. 2016). Non-psychiatric ExAC (version 0.3) variants with genotype quality≥20 and read depth≥10 were used for the above analysis. The genes and variants were then screened against several literature, databases, including Online Mendelian Inheritance in Man (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) were retrieved for 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 5 developmental stages. Amongst 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 + 7 OMIM candidates) genes, and the
remaining 27 genes and their interacting partners, if any, were excluded from the analysis.
The raw data was z-score normalized (gene-wise), and the median absolute deviation (MAD)
(scaling factor, $k=1$) of the expression was calculated for each gene across all 16 tissues in 5
developmental stages. To facilitate the understanding of PPI network dynamics with respect to
the brain regions as well as developmental stages, we developed an open source network
visualisation toolkit. The toolkit is written in JavaScript using ReactJS [https://facebook.github.io/react], SigmaJS [http://sigmajs.org], and D3 [https://d3js.org] packages. This toolkit is accessible publicly on https://placet.noop.pw 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 was integrated into the interactome, with the node sizes representing the expression levels of the corresponding genes. In order to classify the druggable genes from the remaining causative candidates, we employed hierarchical classification of spatio-temporal expression data of 96 SZ genes.

**Post-mortem microarray data analysis:**

Microarray expression profiles of 54675 Affymetrix probesets of PFC (Pre-frontal cortex),
HPC (Hippocampus) and STR (Striatum) from 205 psychiatric subjects (in total) with SZ, BP,
MDD and clinically matched controls were downloaded from GEO (ID: GSE53987) (Lanz et al., 2015). The downloaded data was MAS 5.0 normalized and log2 transformed to make sure that the data follows a Gaussian distribution. The distribution was looked 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 difference in gene expression between cases and controls. The False Discovery Rate (FDR) of t-test p-values was calculated for multiple hypothesis testing. A two fold change (FC>2) in gene expression in cases compared to controls along with a p-value<0.01, was considered to be differentially expressed. Annotation of Affymetrix probe IDs was performed using Affymetrix Netaffx Batch Query (http://www.affymetrix.com/analysis/index.affx). The union
set of all DEGs in 9 different cases was identified. Ganapathiraju et al., had identified 504 novel PPI addition to the 1397 PPI, comprising of 1901 interactions in total. We have arrived at 1718 genes, by identifying the union set of all genes present in 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 SZ candidate genes have been attributed (P<0.01).

Statistical analysis and data visualization:
All the statistical tests and data visualizations were performed using R, including MAS5.0 normalization and statistical corrections of microarray gene expression data.

RESULTS

1. Functional consequences of non-synonymous variants:
In order to characterise the functional implications of the non-synonymous variants in SZ candidate genes, we mined data from Ensembl Variation (EV) (Yates et al., 2015). Out of 123 SZ candidate genes (101 interactome candidate genes + 22 OMIM genes), EV reported 4495 well annotated non-synonymous variants in 100 SZ candidate genes for which the PolyPhen scores were retrieved (Supplementary Table 1A) (Adzhubei et al., 2010).

1.A. Identification and shortlisting of lethal variants using genomes of healthy centenarians and non-psychiatric ExAC database:
According to PolyPhen analysis, it was observed that 2037 (of 4495) variants were lethal (probably damaging) which mapped to 99 (of 100) SZ genes. In order to narrow down the number of deleterious variants, of the 2037 variants, we eliminated 33 variants belonging to
24 genes that were observed in genomes of healthy centenarians (N=93) (Supplementary Table 1B)(http://clingen.igib.res.in/sage/). Of the 2004 variants absent in centenarians (Supplementary Table 1C); i) we found that 265 variants (mapping to 79 genes) were also absent in non-psychiatric ExAC database and were defined as variants of criticality (Supplementary Table 1D) (Exome Aggregation Consortium et al., 2016). ii) we retained the remaining 1739 lethal variants i.e., those absent in centenarians but present in non-psychiatric ExAC, that mapped to 99 genes that could turn deleterious later on under certain circumstances (Supplementary Table 1E). These 1739 variants were further classified into three categories based on their allele frequencies (AF) (AF<0.0001: Personal; AF: 0.0001 to 0.01: Mutational range; AF>0.01: Common). Amongst the 1739 lethal variants, 1319 (mapping to 98 genes) were personal, 405 (mapping to 75 genes) were at 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 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 susceptibility, at the population level. Hence, 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 + 9 genes unique to the mutational range and common variant genes), to contribute to the disease phenotype (Figure 1). Hence, we present a panel of potentially deleterious 685 variants, that could be further investigated for behavioral phenotypes and brain patho-biology in animal models of neuropsychiatric disorders (Supplementary Table 1F). It was also witnessed that, six (CSMD1, CACNA1C, PLCH2, NRG1, ADAMTSL3 and TCF20) out of 88 SZ candidate genes, had a relatively higher burden of non-synonymous variants (>20 variants per gene) (Supplementary Figure 1). It is interesting to note that although the number of variants gets 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 on to a particular locus.

1.B. Distribution of non-synonymous variants present in SZ genes in global populations:
In order to gain an overall snapshot of the allele frequencies of the variants present in SZ genes in global populations, we queried all the original 4495 variants, in non-psychiatric
ExAC database. On analysis, it was observed that 4045 variants were mapped to 99 genes, thereby discarding the 450 variants that were absent in the non-psychiatric ExAC database. The AFs of 4045 variants in 6 populations reported in the non-psychiatric ExAC database, was also retrieved (Supplementary Table 2). The analysis revealed that the number of variants observed in each of the population were directly proportional to their sample size. However, the proportion of the personal variants was higher (n=1843) in the out-bred European population (NFE) but was absent in the inbred Finnish (FIN) and East-Asian Tibeto-Burman (EAS) population (Figure 2). 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 FIN and EAS populations could be an artifact of the under-representation of the corresponding cohorts in the ExAC database.

1.C. Literature mining of variants present in SZ candidate genes that have been associated with multiple chronic illnesses:
In order 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 non-synonymous variants present in 37 SZ genes, i.e., almost 40% of all schizophrenia candidates (Amberger et al., 2015). These variants were associated with other disorders include BP, MDD, Autism, Epilepsy, Seizure, Alzheimer’s, diabetes, hypertension, etc (Supplementary Table 3). Amongst 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. Out of these 22 presumed lethal variants, twelve (rs2904552, rs3970559, rs34845648, rs8192466, rs45571736, rs2229961, rs34622148, rs1801500, rs769455, rs1801158, rs3970559 and rs2904552) were found to be absent in centenarian but present in the non-psychiatric ExAC database. However, none of the 22 were absent both in the centenarians and the non-psychiatric ExAC database.

2. Analysis of Spatio-Temporal Interactome:
Although the PPI map for SZ (Ganapthiraju et al., 2016) presented all the possible interactions, a large proportion of the genes represented in the interactome are not co-expressed 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 (5 developmental stages) (http://placet.noop.pw/) (Supplementary Table 4; Supplementary Figure 2) (Tebbenkamp et al., 2014; Grote et al., 2016).

2.A. Extent of difference in gene expression between hub genes and non-hub genes:

Hyperlink-Induced Topic Search (HITS) was used to rank the genes as hubs (top 10 genes) or non-hubs (bottom 10 genes). With this study, we hypothesized that highly connected genes i.e., the hub genes, must be expressed significantly higher compared to non-hub genes in-order to interact with larger set of proteins. However, no difference was found between the gene expression means of hub genes and non-hub genes.

2.B. Characterization of gene expression dynamics in regions of adolescent and adult brain:

In order to identify the genes that exhibit high variations in expression pattern in a normal human brain, we carried out median absolute deviation (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 amongst 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 (Supplementary Table 5).

2.C. Expression based similarity search for druggable SZ candidate genes:

Despite large GWAS findings, it still remains unclear which, if any, of the newly identified GWAS loci will serve as good starting points for drug development in SZ (Dolgin et al., 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. In order to classify the druggable genes from the remaining causative candidates we employed hierarchical classification of spatio-temporal expression data of 96 SZ candidate genes and identified a sub-cluster of 11 genes (IGSF9B, NAB2, DAO, CYP26B1, PLCH2, CHRNA3, SLC6A3, DRD2, DRD3, DAOA and TAAR6), which were enriched only in certain regions of the brain at certain developmental stages (Figure 3).
Amongst the sub-cluster of 11 genes, three (SLC6A3, DRD2 and DRD3) have been well-established drug targets for SZ (Figure 4). SLC6A3 was found to be active in child and adolescent thalamus, a region which has been implicated in SZ (Figure 4C) (Alelú-Paz et al., 2008; Pergola et al., 2015). DRD2 (Figure 4A) and DRD3 (Figure 4B) were found to be active only in striatum across all developmental stages. Striatum has been associated with the pathophysiology of SZ, BP, ASD and adolescent depression (Brunelin et al., 2016; Simpson et al., 2010; Fuccillo et al., 2016; Caseras et al., 2013; Gabbay et al., 2013). DAO and DAOA which also belong to the sub-cluster, have been receiving attention as potential alternative therapeutic means to enhance NMDAR function in SZ patients (Verrall et al., 2010; Sehgal et al., 2015). DAO was found to be enriched in anterior cingulate cortex (MFC) which has also been implicated in SZ (Pomarol-Clotet et al., 2010). Interactors of NAB2 are targeted by nervous system drugs in the management of epilepsy, which often co-occurs with SZ (Ganapthiraju et al., 2016; Cascella et al., 2009). NAB2 is highly expressed in cerebellar cortex, a brain region that shows modest association with SZ endophenotypes (Kim et al., 2014; Bang et al., 2018; Andreasen et al., 2011). Trace amine-associated receptors (TAAR) are GPCRs that are often activated after the blockade of dopaminergic receptors by antipsychotics (Kleinau et al., 2011). It is also interesting to note the silence of TAAR6 in most regions of the brain which are of interest in SZ, except in the MFC (Child). Whether TAAR activation observed is a pathobiological correlate of the disorder, or a consequence of receptor blockade by antipsychotics, and thus a psycho pharmacologically mediated process, needs to be understood. Although the expression of the remaining 4 genes (IGSF9B, CYP26B1, PLCH2 and CHRNA3) in the sub-cluster show druggability signatures, no strong evidence was available from existing literature. The spatio-temporal expression maps of 96 SZ genes are available in Supplementary File 2. Multiple evidences indicate that the therapeutic outcome of antipsychotics is mediated not just by the classical dopamine D2/D3 receptors, but also other targets including the D1 receptors. Moreover, the clinical outcome i.e., full or partial recovery from the first incidence of psychotic episodes may take weeks or even months, although the relevant receptors are blocked within hours (Miller et al., 2009). This multitude of effects, and side effects, and the latency of clinical outcome suggests a complex interplay of multiple biological pathways. These processes needs to be understood using post-mortem gene expression data, as well as data from animal model systems, to able to identify potential alternative drug targets for psychiatric disorders.
2.D. Analysis of differentially expressed genes in psychiatrically ill post-mortem brain tissues and their overlap with SZ interactome:

In order 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 were examined (Lanz et al., 2015). Analysis of expression profiles of PFC, HPC and STR revealed 985 unique DEGs (FC>2; \( P<0.01 \)) from nine different conditions (Supplementary File 3). The raw t-test \( P \)-values were used since FDR corrected \( P \)-values were not significant for most genes to be called as differentially expressed. In order to validate the role of genes from the SZ interactome (Ganapthiraju et al., 2016) in post-mortem brain tissues, we overlapped the gene IDs of 985 DEGs and 1718 genes in SZ interactome. We obtained an overlap of 71 genes (2 candidate genes + 69 interactors) that were present in the SZ interactome and also differentially expressed in post-mortem brain tissues; of which 22 being novel interactors as predicted by Ganapthiraju et al., 2016. Fourteen of the 22 dysregulated genes in our analysis revealed direct or indirect relationships with neuropsychiatric disorders and co-morbidities from previous studies (Table 1). The remaining 8 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. On analysis, it was observed that only two SZ candidate genes (SLC6A4 and CACNB2) were differentially expressed in HPC (BP) (log2FC=1.31; \( P=0.005 \)) and PFC (MDD) (log2FC=-1.11; \( P=0.008 \)) respectively (Supplementary File 3). Interestingly, 14 out of 71 DEGs were previously identified as druggable targets of various FDA approved drugs in the gene-drug interactome study by Ganapthiraju et al., 2016. The above analysis is illustrated in Supplementary Figure 3.

3. Analysis of druggable genes and pathways:

A 2-D matrix representing 286 biological pathways involving 122 druggable genes was reconstructed from the literature (Ganapthiraju et al., 2016). From the above post mortem gene expression data from patients, we identified 14 druggable DEGs. Of these 14 DEGs, four (PTGS1, ERBB2, PTGER3 and ESR2) were found to be downregulated in at least one of the nine conditions. Since, drug induced inhibition of highly expressed would be easier (It’s all druggable., 2017), 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) (Supplementary File 4) belonging to 81 biological pathways (Supplementary Table 6).

3.A. Investigation into druggable genes present in pathways belonging to SZ candidate genes:

Amongst 71 DEGs identified from post-mortem brains, only 2 were candidate genes (SLC6A4 and CACNB2), and 10 were druggable interactors. In order to identify more druggable genes upstream or downstream in the biological pathways associated with SZ, we used ConsensusPathDB (CPDB) (Kamburov et al., 2011) 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 (out of 123) genes in 54 biological pathways which includes Dopaminergic signaling, MAPK signaling, cAMP signaling, Axon guidance, Calcium signaling, Gaα signaling, Celecoxib pharmacodynamics, T-cell receptor signaling, Alzheimer’s disease pathway, etc (Supplementary Table 7). Of these 54, only 3 pathways had putative drug targets (n=4 i.e., COX-2, GNAQ, PLCB1 and PDE10A) and their spatio-temporal expression (Z-score_RPKM) profiles are also provided (Supplementary Figure 4,5,6,7).

3.A.1. Celecoxib pharmacodynamics pathway:

Celecoxib, a COX-2 inhibitor is an anti-inflammatory drug used to treat osteoarthritis, RA, Ankylosing spondylitis (AS), acute pain in adults and juvenile arthritis. It has also been investigated as an adjuvant in several psychiatric disorders like MDD, BP and SZ (Fan et al., 2013; Muller et al., 2008; Na et al., 2014; Rosenblat et al., 2014; Fond et al., 2014). On analysis, the SZ genes, CACNB2, PTGIS, ATP2A2 and AKT1 were found to be involved in Celecoxib pharmacodynamics. Although the expression of the above over-represented genes has been characterized in previous analysis, the spatio-temporal enrichment of COX-2 with respect to celecoxib metabolism has never been reported. Spatio-temporal expression profiles of COX-2 were retrieved from BrainSpan atlas database using ABAEnrichment. Further analysis revealed that COX-2 levels were high in all the 11 cortical regions of Infant, Child and Adolescent human brain, especially in Infant (S1C) and Adolescent (A1C) (Supplementary Figure 4).
3.A.2. Ga signaling pathway:
The associations of G-protein coupled receptors and Ga subunits in phosphoinositide signaling with psychoses has been well established (Catapano et al., 2007; Rajy et al., 1997). The two genes (PLCB1 and GNAQ) were found to be involved in Ga signaling pathway and deletions in the former were observed in SZ patients (Lo et al., 2013). The analysis revealed that the expression of GNAQ was high in Prenatal (CBC) (Supplementary Figure 5). However, expression of PLCB1 was found to be high in V1C (Adult, Child and Infant) and Infant (STR) (Supplementary Figure 6).

3.A.3. cGMP-PKG signaling pathway:
Phosphodiesterase 10A (PDE10A) is a basal ganglia specific hydrolase that regulates cAMP/cGMP signaling cascades. Animal studies have revealed that PDE10A inhibitors could provide efficacy on the positive and negative symptoms of SZ, and these are currently being evaluated in clinical trials (Kehler et al., 2011). However, their spatio-temporal expression patterns were unknown. Our analysis using BrainSpan data revealed that PDE10A was enriched only in STR, which is a part of basal ganglia. Maximum expression of PDE10A was observed in infant (STR) (Supplementary Figure 7).

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 amongst which several were targets of FDA approved drugs (Ganapthiraju et al., 2016). With the availability of the genomes of healthy centenarians, non-psychiatric 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 centenerian genomes and non-psychiatric 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. Further, in
order to identify the genes present in the SZ interactome that are dysregulated in psychiatric
patients, the microarray expression profiles from 205 post-mortem brains were looked into,
and the DEGs were overlapped with the union set of all genes present in the SZ interactome.
Twenty two novel interactors present in the SZ interactome were found to be dysregulated in
post-mortem brains. These proteins previously had null or minimal associations with
psychoses, thereby now validating a subset of the novel interactors as predicted by
Ganapthiraju 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
schizophrenia cases (Table 1) (McDonald et al., 1998). Although no direct evidence for
psychoses was found for 8 novel interactors that were dysregulated in psychiatric post-
mortem brains, some of them (MYOZ2, GSC2, MKI67 and VANGL1) were discernable 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 signaling, which are critical for SZ biology (Lidow et al., 2003; Miyakawa et al.,
2003). GSC2, a homeodomain containing gene resides on 22q11, which is a hotspot for
psychoses (Saleem Q 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 MS 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 repurposition (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 non-receptor targets (ACE, mTOR, PTPN1, NOS3 and CFTR),
in ameliorating the whole spectrum of symptoms of SZ and other psychoses. 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. The biological pathways,
though diverse, cover a broader spectrum of cellular functions such as viability, proliferation and regulation of cell motility, which are generic, but may be critical to the pathobiology of schizophrenia. 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 for 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 cells (iPSC) derived neuronal lineages of SZ patients (Viswanath et al., 2018), could be useful to help unravel the biology of mental illness, and also accelerating the drug repurposing pipelines.

**Availability of data and materials:**
This spatio-temporal dynamic network is accessible publicly on [https://placet.noop.pw](https://placet.noop.pw) and the Source code can be accessed from [https://github.com/prashnts/placet](https://github.com/prashnts/placet). All PolyPhen annotated variants, DEGs identified from post-mortem microarray, lists of biological pathways etc., are available as Supplementary Files.

**AUTHOR DISCLOSURE**

**Role of the funding source**
No extramural funding was availed to carry out the project.

**Contributors**
SKB conceptualized and designed the project. ACS and AKJ performed the gene expression data analysis. AKP performed the non-psychiatric ExAC variation analysis 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.

**Conflict of Interest**
The authors declare no conflict of interest.
Acknowledgments

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 unpublished centenarian genome data and Beena Pillai for inputs on gene expression data analysis. We finally thank Meera Purushottam, Biju Viswanath and Ravi Kumar Nadella for critical reading of the manuscript.

REFERENCES

1. AbdAlla S et al., ACE Inhibition with Captopril Retards the Development of Signs of Neurodegeneration in an Animal Model of Alzheimer’s Disease. Int. J. Mol. Sci. 2013, 14(8), 16917-16942.
2. Adzhubei, Ivan A., et al. A method and server for predicting damaging missense mutations. Nature methods 7.4 (2010): 248-249.
3. Alelú-Paz, Raúl, and José Manuel Giménez-Amaya. The Mediodorsal Thalamic Nucleus and Schizophrenia. Journal of Psychiatry & Neuroscience : JPN 33.6 (2008): 489–498.
4. Amberger, JS et al. A. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Research. 2015;43(Database issue):D789-D798.
5. Andreasen, Nancy C. et al. Progressive Brain Change in Schizophrenia: A Prospective Longitudinal Study of First-Episode Schizophrenia. Biological Psychiatry 70.7 (2011): 672–679.
6. Bang, M et al. Aberrant cerebro-cerebellar functional connectivity and minimal self-disturbance in individuals at ultra-high risk for psychosis and with first-episode schizophrenia. Schizophr Res. 2018 Jun 18. pii: S0920-9964(18)30365-7.
7. Brunelin, J et al. Abnormal Striatal Dopamine Transmission in Schizophrenia. Current Medicinal Chemistry 20.3 (2013): 397–404.
8. Cardno, Alastair G et al. A Twin Study Of Schizoaffective-Mania, Schizoaffective-Depression And Other Psychotic Syndromes. American Journal of Medical Genetics 159B.2 (2012): 172–182.

9. Carty NC, Xu J, Kurup P, et al. The tyrosine phosphatase STEP: implications in schizophrenia and the molecular mechanism underlying antipsychotic medications. Translational Psychiatry. 2012;2(7):e137.

10. Cascella, Nicola G., David J. Schretlen, and Akira Sawa. SCHIZOPHRENIA AND EPILEPSY: IS THERE A SHARED SUSCEPTIBILITY? Neuroscience research 63.4 (2009): 227–235.

11. Caseras, Xavier et al. Ventral Striatum Activity in Response to Reward: Differences Between Bipolar I and II Disorders. The American Journal of Psychiatry 170.5 (2013): 533–541.

12. Castellani, Christina A et al. DNA Methylation Differences in Monozygotic Twin Pairs Discordant for Schizophrenia Identifies Psychosis Related Genes and Networks. BMC Medical Genomics 8 (2015): 17.

13. Catapano, Lisa A., and Husseini K. Manji. G Protein-Coupled Receptors in Major Psychiatric Disorders. Biochimica et biophysica acta 1768.4 (2007): 976–993.

14. Dal Pra, I et al. Do astrocytes collaborate with neurons in spreading the "infectious" aβ and Tau drivers of Alzheimer's disease? Neuroscientist. 2015 Feb;21(1):9-29.

15. De Peri, Luca, et al. Brain structural abnormalities at the onset of schizophrenia and bipolar disorder: a meta-analysis of controlled magnetic resonance imaging studies. Current pharmaceutical design 18.4 (2012): 486-494.

16. Dolgin, E. Massive Schizophrenia Genomics Study Offers New Drug Directions. Nature Reviews Drug Discovery 13.9 (2014): 641-642.

17. Eckman, EA et al. Regulation of steady-state beta-amyloid levels in the brain by neprilysin and endothelin-converting enzyme but not angiotensin-converting enzyme. The Journal of Biological Chemistry (2006): 281(41):30471-8.

18. Effectiveness and tolerance of anti-inflammatory drugs' add-on therapy in major mental disorders: a systematic qualitative review.

19. Exome Aggregation Consortium et al. Analysis of Protein-Coding Genetic Variation in 60,706 Humans. Nature 536.7616 (2016): 285–291.
20. Fan, Xiaoduo, and Xueqin Song. Non-steroidal anti-inflammatory drugs may reduce schizophrenia symptom severity in the short term when added to antipsychotics. (2013).

21. Farrell M, Werge T, Sklar P, et al. Evaluating Historical Candidate Genes for Schizophrenia. Molecular psychiatry. 2015;20(5):555-562. doi:10.1038/mp.2015.16.

22. Fillman et al. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. Molecular Psychiatry (2013): 18(2):206-14.

23. Freedman R et al. Evidence for the multigenic inheritance of schizophrenia. American Journal of Medical Genetics (2001): 105(8):794-800.

24. Fuccillo, Marc V. Striatal Circuits as a Common Node for Autism Pathophysiology. Frontiers in Neuroscience 10 (2016): 27.

25. Gabbay, Vilma et al. Striatum-Based Circuitry of Adolescent Depression and Anhedonia. Journal of the American Academy of Child and Adolescent Psychiatry 52.6 (2013): 628–41.e13.

26. Gadelha A et al. Convergent evidences from human and animal studies implicate angiotensin I-converting enzyme activity in cognitive performance in schizophrenia. Translational Psychiatry (5), page e691 (2015).

27. Gadelha, A et al. Angiotensin converting enzyme activity is positively associated with IL-17a levels in patients with schizophrenia. Psychiatry Research. (2015): 702-7.

28. Gadelha, A et al. Convergent Evidences from Human and Animal Studies Implicate Angiotensin I-Converting Enzyme Activity in Cognitive Performance in Schizophrenia. Translational Psychiatry 5.12 (2015): e691–. PMC. Web. 8 Oct. 2017.

29. Ganapathiraju, Madhavi K., et al. Schizophrenia interactome with 504 novel protein–protein interactions. npj Schizophrenia 2 (2016): 16012.

30. Gibbons, AS et al. Low Density Lipoprotein Receptor-Related Protein and Apolipoprotein E Expression is Altered in Schizophrenia. Frontiers in Psychiatry. 2010;1:19. doi:10.3389/fpsyt.2010.00019.

31. Gibbons, Andrew Stuart et al. “Low Density Lipoprotein Receptor-Related Protein and Apolipoprotein E Expression Is Altered in Schizophrenia.” Frontiers in Psychiatry 1 (2010): 19.

32. Gillen AE and Harris A. Transcriptional regulation of CFTR gene expression. Frontiers in Bioscience (2012): 4:587-92.
33. Girard, Simon L., Patrick A. Dion, and Guy A. Rouleau. Schizophrenia genetics: putting all the pieces together. Current neurology and neuroscience reports 12.3 (2012): 261-266.

34. Grote, Steffi, et al. ABAEnrichment: an R package to test for gene set expression enrichment in the adult and developing human brain. Bioinformatics 32.20 (2016): 3201-3203.

35. Gupta S, Bisht SS, Kukreti R, Jain S and Brahmachari SK. Boolean network analysis of a neurotransmitter signaling pathway. Journal of Theoretical Biology (2007): 244(3):463-9.

36. Haybaeck, J et al. Increased expression of retinoic acid-induced gene 1 in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, and major depression. Neuropsychiatric Disease and Treatment. 2015;11:279-289.

37. Haybaeck, Johannes et al. Increased Expression of Retinoic Acid-Induced Gene 1 in the Dorsolateral Prefrontal Cortex in Schizophrenia, Bipolar Disorder, and Major Depression. Neuropsychiatric Disease and Treatment 11 (2015): 279–289.

38. He, R et al. Protein tyrosine phosphatases as potential therapeutic targets. Acta Pharmacologica Sinica. 2014;35(10):1227-1246.

39. Hendy GN et al. The CASR gene: alternative splicing and transcriptional control, and calcium-sensing receptor (CaSR) protein: structure and ligand binding sites. Best Practice & Research Clinical Endocrinology & Metabolism (2013): 27(3):285-301.

40. Hindorff, Lucia A., et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences 106.23 (2009): 9362-9367.

41. Hobgood DK. ACE inhibitors could be therapeutic for antisocial personality disorder. Med Hypotheses. 2013 Nov;81(5):757-9.

42. Hoosain FG, Choonara YE, Tomar LK, et al. Bypassing P-Glycoprotein Drug Efflux Mechanisms: Possible Applications in Pharmacoresistant Schizophrenia Therapy. BioMed Research International. 2015;2015:484963. doi:10.1155/2015/484963.

43. Howard, Robert M.D, et al. Late-onset schizophrenia and very-late-onset schizophrenia-like psychosis: an international consensus. The International Late-Onset Schizophrenia Group. American Journal of Psychiatry (2000): 172-8.
44. Imming P, Sinning C and Meyer A. Drugs, their targets and the nature and number of drug targets. Nature Reviews Drug Discovery (2006): 5(10):821-34.
45. It’s all druggable, Nature Genetics. 2017
46. Jarvis CI, Goncalves MB, Clarke E, et al. Retinoic acid receptor-α signalling antagonizes both intracellular and extracellular amyloid-β production and prevents neuronal cell death caused by amyloid-β. The European Journal of Neuroscience. 2010;32(8):1246-1255.
47. Kamburov, Atanas et al. ConsensusPathDB: Toward a More Complete Picture of Cell Biology. Nucleic Acids Research 39.Database issue (2011): D712–D717.
48. Kariuki, Silvia N. et al. Promoter Variant of PIK3C3 Is Associated with Autoimmunity against Ro and Sm Epitopes in African-American Lupus Patients. Journal of Biomedicine and Biotechnology 2010 (2010): 826434.
49. Kataoka, M et al. Exome Sequencing for Bipolar Disorder Points to Roles of de Novo Loss-of-Function and Protein-Altering Mutations. Molecular Psychiatry 21.7 (2016): 885–893.
50. Kehler J and Nielsen J. PDE10A inhibitors: novel therapeutic drugs for schizophrenia. Current Pharmaceutical design (2011): 17(2):137-50.
51. Keshavan MS, Anderson S and Pettegrew JW. Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? The Feinberg hypothesis revisited. Journal of Psychiatric Research. (1994): 239-65.
52. Kibar, Zoha et al. Novel Mutations in VANGL1 in Neural Tube Defects. Human Mutation 30.7 (2009): E706–E715.
53. Kim D-J, Kent JS, Bolbecker AR, et al. Disrupted Modular Architecture of Cerebellum in Schizophrenia: A Graph Theoretic Analysis. Schizophrenia Bulletin. 2014;40(6):1216-1226.
54. Kim DH et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell (2002): 110(2):163-75.
55. Kim JY, Ho H, Kim N, et al. Calcium-sensing receptor (CaSR) as a novel target for ischemic neuroprotection. Annals of Clinical and Translational Neurology. 2014;1(11):851-866. doi:10.1002/acn3.118.
56. Kim, Eunhee et al. Role of Spleen-Derived Monocytes/macrophages in Acute Ischemic Brain Injury. Journal of Cerebral Blood Flow & Metabolism 34.8 (2014): 1411–1419.

57. Kleinau, Gunnar et al. “From Molecular Details of the Interplay between Transmembrane Helices of the Thyrotropin Receptor to General Aspects of Signal Transduction in Family A G-Protein-Coupled Receptors (GPCRs).” The Journal of Biological Chemistry 286.29 (2011): 25859–25871.

58. Kumar, V. et. al. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. J. Neuroscience. 25, 11288-11299 (2005).

59. Kumar, Vinod et al. Systematic Analysis of Drug Targets Confirms Expression in Disease-Relevant Tissues. Scientific Reports 6 (2016): 36205.

60. Lafionatiatis A et al. Effects of the inducible nitric oxide synthase inhibitor aminoguanidine in two different rat models of schizophrenia. Behav Brain Res. 2016 Aug 1;309:14-21

61. Lanz, TA et al. STEP levels are unchanged in pre-frontal cortex and associative striatum in post-mortem human brain samples from subjects with schizophrenia, bipolar disorder and major depressive disorder. PLos One (2015): 10(3): e0121744.

62. Lehtinen V. Prevalence of mental disorders among adults in Finland: basic results from the Mini Finland Health Survey. Acta Psychiatria Scandinavica (1990): 81(5):418-25.

63. Lerner V, McCaffery PJ and Ritsner MS. Targeting Retinoid Receptors to Treat Schizophrenia: Rationale and Progress to Date. CNS Drugs (2016): 269-80.

64. Lidow MS. Calcium signaling dysfunction in schizophrenia: a unifying approach. Brain Research: Brain research reviews (2003): 43(1):70-84.

65. Lipton and Sahin., The neurology of mTOR. Neuron. 2014 Oct 22;84(2):275-91

66. Lo, V et al. Phosphoinositide-Specific Phospholipase C β1 Gene Deletion in Bipolar Disorder Affected Patient. Journal of Cell Communication and Signaling 7.1 (2013): 25–29.

67. Malaspina A and Michael-Titus. Is the modulation of retinoid and retinoid-associated signaling a future therapeutic strategy in neurological trauma and neurodegeneration? J Neurochem. 2008 Feb;104(3):584-95. Epub 2007 Nov 22.
68. Marsden PA et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. Journal of Biological Chemistry (1993): 268(23):17478-88.

69. McCullumsmith, Robert E et al. Expression of Transcripts for Myelination-Related Genes in the Anterior Cingulate Cortex in Schizophrenia. Schizophrenia Research 90.1-3 (2007): 15–27.

70. McDonald C et al. Retinitis pigmentosa and schizophrenia. (1998): 13(8):423-6

71. McGrath et al. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 2008;30:67-76.

72. Miller, R. “Mechanisms of Action of Antipsychotic Drugs of Different Classes, Refractoriness to Therapeutic Effects of Classical Neuroleptics, and Individual Variation in Sensitivity to Their Actions: PART I.” Current Neuropharmacology 7.4 (2009): 302–314. PMC. Web. 30 Mar. 2018.

73. Miyakawa, Tsuyoshi et al. Conditional Calcineurin Knockout Mice Exhibit Multiple Abnormal Behaviors Related to Schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 100.15 (2003): 8987–8992.

74. Mokhtari, Ryan, and Herbert M Lachman. The Major Histocompatibility Complex (MHC) in Schizophrenia: A Review. Journal of clinical & cellular immunology 7.6 (2016): 479.

75. Muller, Norbert, and Markus J. Schwarz. COX-2 inhibition in schizophrenia and major depression. Current pharmaceutical design 14.14 (2008): 1452-1465.

76. Na, Kyoung-Sae, et al. Efficacy of adjunctive celecoxib treatment for patients with major depressive disorder: a meta-analysis. Progress in Neuro-psychopharmacology and Biological Psychiatry 48 (2014): 79-85.

77. Nadalin S et al. The insertion/deletion polymorphism in the angiotensin-converting enzyme gene and nicotine dependence in schizophrenia patients. Journal of Neural Transmission. (2017): 511-518.

78. Naheed, Miriam, and Ben Green. Focus on clozapine. Current medical research and opinion 17.3 (2001): 223-229.

79. Nakazawa et al. Differential gene expression profiles in neurons generated from lymphoblastoid B-cell line-derived iPS cells from monozygotic twin cases with
treatment-resistant schizophrenia and discordant responses to clozapine. Schizophrenia Research (2017): 181:75-82.

80. Narayanaswamy JC, Viswanath B and Bada Math S. Schizophrenia and retinitis pigmentosa: are there mechanisms which blind insanity? European Psychiatry (2013): 47(1):95-6.

81. Nourooz-Zadeh, J et al. Low-Density Lipoprotein Is the Major Carrier of Lipid Hydroperoxides in Plasma. Relevance to Determination of Total Plasma Lipid Hydroperoxide Concentrations. Biochemical Journal 313.Pt 3 (1996): 781–786.

82. Pergola G et al. The role of the thalamus in schizophrenia from a neuroimaging perspective. Neurosci Biobehav Rev. 2015 Jul;54:57-75.

83. Pham, X et al. The DPYSL2 Gene Connects mTOR and Schizophrenia. Translational Psychiatry 6.11 (2016): e933

84. Phillippe Kastner and Susan Chan. Function of RARalpha during the maturation of neutrophils. Oncogene (2001): 20(49):7178-85.

85. Pilar Saiz. Association Study Of Endothelial Nitric Oxide Synthase (NOS3) Gene Polymorphisms And Schizophrenia. Schizophrenia Research (2008): 102:1-3.

86. Pitsikas N. The Role of Nitric Oxide Synthase Inhibitors in Schizophrenia. Curr Med Chem. 2016;23(24):2692-2705.

87. Pomarol-Clotet, E et al. Medial Prefrontal Cortex Pathology in Schizophrenia as Revealed by Convergent Findings from Multimodal Imaging. Molecular Psychiatry 15.8 (2010): 823–830.

88. Ponta H, Sherman L and Herrlich PA. CD44: from adhesion molecules to signalling regulators. Nature Reviews Molecular Cell Biology (2003): 4(1):33-45.

89. Puljak L and Kilic G. Emerging roles of chloride channels in human diseases. Biochim Biophys Acta. 2006 Apr;1762(4):404-13. Epub 2006 Jan 17.

90. Rajy Mathews et al. Increased Gaq/11 immunoreactivity in post-mortem occipital cortex from patients with bipolar affective disorder. Biological Psychiatry (1997): 649–656.

91. Rioux L, Arnold SE. The expression of retinoic acid receptor alpha is increased in the granule cells of the dentate gyrus in schizophrenia. Psychiatry Res. 2005 Jan 30;133(1):13-21.
92. Ripke et al., Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511.7510 (2014): 421-427.

93. Rogers, Jonathan, and Matthew J. Taylor. Pharmacological agents to reduce readmissions in bipolar disorder. (2017): 387-388.

94. Rosenblat, Joshua D., et al. Inflamed moods: a review of the interactions between inflammation and mood disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry 53 (2014): 23-34.

95. SAGE - South Asian Genomes and Exomes, http://clingen.igib.res.in/sage/.

96. Saito S et al. An association study of tachykinin receptor 3 gene with schizophrenia in the Japanese population. Neureport (2008): 19(4):471-3.

97. Saleem, Q et al. Association of CAG repeat loci on chromosome 22 with schizophrenia and bipolar disorder. Molecular Psychiatry (2001): 694-700.

98. Sanders, A R et al. Transcriptome Sequencing Study Implicates Immune-Related Genes Differentially Expressed in Schizophrenia: New Data and a Meta-Analysis. Translational Psychiatry 7.4 (2017): e1093.

99. Sehgal, Sheikh Arslan et al. Adaptive Evolution and Elucidating the Potential Inhibitor against Schizophrenia to Target DAOA (G72) Isoforms. Drug Design, Development and Therapy 9 (2015): 3471–3480.

100. Serretti A and Mandelli L. The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions. Molecular Psychiatry (2008): 13(8):742-71.

101. Shen, WW. A history of antipsychotic drug development. Comprehensive Psychiatry (1999): 407-14.

102. Shinkai, T et al. Allelic association of the neuronal nitric oxide synthase (NOS1) gene with schizophrenia. Molecular Psychiatry (2002): 560-3.

103. Simpson, EH et al. A Possible Role for the Striatum in the Pathogenesis of the Cognitive Symptoms of Schizophrenia. Neuron 65.5 (2010): 585–596.

104. Soudais C et al. Independent mutations of the human CD3-epsilon gene resulting in a T cell receptor/CD3 complex immunodeficiency. Nature Genetics (1993): 3(1):77-81.
105. Sušilová, Lenka, et al. Changes in BMI in hospitalized patients during treatment with antipsychotics, depending on gender and other factors. International Journal of Psychiatry in Clinical Practice 21.2 (2017): 112-117.

106. Takahashi, Sakae et al. Association of SNPs and Haplotypes in APOL1, 2 and 4 with Schizophrenia. Schizophrenia Research 104.0 (2008): 153–164.

107. Tang R et al. Investigation of variants in the promoter region of PIK3C3 in schizophrenia. Neuroscience Letters (2008): 437(1):42-4.

108. Tebbenkamp, Andrew TN, et al. The developmental transcriptome of the human brain: implications for neurodevelopmental disorders. Current opinion in neurology 27.2 (2014): 149.

109. Verrall, L et al. The Neurobiology of D-Amino Acid Oxidase (DAO) and Its Involvement in Schizophrenia. Molecular psychiatry 15.2 (2010): 122–137.

110. Viswanath B, Rao NP, Narayananswamy JC, et al. Discovery biology of neuropsychiatric syndromes (DBNS): a center for integrating clinical medicine and basic science. BMC Psychiatry. 2018;18:106.

111. Wang, Long et al. Brain Development and Akt Signaling: The Crossroads of Signaling Pathway and Neurodevelopmental Diseases. Journal of Molecular Neuroscience 61.3 (2017): 379–384.

112. Wass C et al. Nitric oxide synthase inhibition attenuates phencyclidine-induced disruption of cognitive flexibility. Pharmacol Biochem Behav. 2008 May;89(3):352-9.

113. Wong, M-L et al. Polymorphisms in Inflammation-Related Genes Are Associated with Susceptibility to Major Depression and Antidepressant Response. Molecular Psychiatry 13.8 (2008): 800–812.

114. Yates, A., et al. (2015). Ensembl 2016. Nucleic acids research, 44(D1), D710-D716.

115. Yin, X et al. Integration of expression quantitative trait loci and pleiotropy identifies a novel psoriasis susceptibility gene, PTPN1. The Journal of Gene Medicine. (2017): 1-2

116. Zammit, Stanley et al. Schizophrenia and Neural Tube Defects: Comparisons From an Epidemiological Perspective. Schizophrenia Bulletin 33.4 (2007): 853–858.

117. Zelinger, Lina et al. A Missense Mutation in DHDDS, Encoding Dehydrodolichyl Diphosphate Synthase, Is Associated with Autosomal-Recessive Retinitis Pigmentosa in Ashkenazi Jews. American Journal of Human Genetics 88.2 (2011): 207–215.
118. Zhang, Chen, et al. Metabolic adverse effects of olanzapine on cognitive dysfunction: A possible relationship between BDNF and TNF-alpha. Psychoneuroendocrinology 81 (2017): 138-143.

119. Zhou M, Li W, Huang S, et al. mTOR Inhibition Ameliorates Cognitive and Affective Deficits Caused by Disc1 Knockdown in Adult-Born Dentate Granule Neurons. Neuron. 2013;77(4):647-654.

FIGURE LEGENDS

Figure 1: Workflow: Multi-scale analysis of SZ genes

Figure 2: Population distribution of personal, mutational range and common non-synonymous variants in SZ genes from non-psychiatric ExAC database. Amongst 4495 variants, 4045 mapping to 99 (out of 100 SZ genes) were identified in 6 populations. The variants observed in each population are directly proportional to their sample size. The bar diagram represents personal variants in blue color, the mutational range variants in red and the common variants in yellow color. AFR: African/African American; AMR: Latino; EAS: East Asian; FIN: Finnish; NFE: Non-Finnish European; SAS: South Asians.

Figure 3: 11 druggable candidate genes classified based on similarity in gene expression signatures. Blue: known drug targets for SZ; Red: putative drug targets for SZ. Amongst which SLC6A3, DRD2, DRD3, DAO, DAOA and CHRNA3 involve in neurotransmission.

Figure 4: Spatio-temporal expression profiles (Z_score RPKM) of druggable SZ candidate genes A.) DRD2, B.) DRD3 and C.) SLC6A3 in a developing human brain.
| Gene_ID   | Disorder (Tissue) | log₂FC | 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                                                   | Katoka et al., 2016         |
|           | MDD (PFC)        | 1.08   | 0.004   |                                                                                                 |                             |
| GNAS      | SZ (HPC)         | -1.65  | 0.003   | Differentially methylated region (DMR) 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 | Zelinger et al., 2011       |
|           |                  |        |         |                                                                                                 | McDonald et al., 1998       |
|           |                  |        |         |                                                                                                 | Narayanaswamy et al., 2013  |
| CD44      | SZ (STR)         | 1.03   | 0.006   | Upregulated in SZ postmortem DFC                                                                 | Fillman et al., 2013        |
| ATP6V0A2  | BP (PFC)         | 1.25   | 0.005   | Mutations in same region repeatedly linked with BP                                               | Serretti et al., 2008       |
| ERAP2     | BP (PFC)         | -1.09  | 0.004   | A functional variant (rs3813065/-442 C/T) on PIK3C3 gene which regulated the expression of ERAP2 was associated with increased risk to SZ in Chinese individuals. | Kariuki et al., 2010        |
|           |                  |        |         |                                                                                                 | Tang et al., 2008           |
| 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            |
| AGFI1G1   | BP (STR)         | 1.11   | 0.008   | Upregulated in SZ lymphoblastoid cell lines                                                     | Sanders et al., 2017        |
| PPP1R1I1  | MDD (HPC)        | -1.36  | 0.008   | Resides within MHC class 1 loci, SZ hotspot                                                      | Mokhtari et al., 2016       |
| TACR3     | MDD (HPC)        | -1.61  | 0.001   | Insignificant association for genotype/haplotype markers in Japanese populations                 | Saito et al., 2008          |

Table 1: Dysregulated novel interactors identified from post-mortem microarray analysis.
| Drug Target (Gene symbol) (n=10) | Gene name | Interacting drugs (FDA approved) | No. of FDA approved drugs (n=34) | Relevance to psychoes/brain biology | Support for druggability (animal models, etc) |
|---------------------------------|-----------|----------------------------------|---------------------------------|-----------------------------------|-----------------------------------------------|
| ACE                             | Angiotensin converting enzyme | Ramipril, Fosinopril, Trandolapril, Benzopril, Enalapril, Moexipril, Perindopril, Quinapril, Rescinnamine, Captopril, Cilazapril, Spirapril and Temocapril | 13                               | Nadalin et al., 2017 Gadelha et al., 2015 Gadelha et al., 2015 Eckman et al., 2006 | OK Hobgood, 2013 AbdiAlla S et al. 2013 Gadelha A et al. 2015 |
| 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 Lipton ans Sahin. 2014. Zhou et al. 2013 |
| RARA                            | Retinoic acid receptor alpha | Acitretin, Adapalene, Tazarotene, Ablretinoin and Etretinate | 5                               | Phillippe et al., 2001 Haybaecck et al., 2015 Lerner et al., 2016 | Malaspina 2008. Jarvis et al., 2010 Haybaecck et al. 2015 Rioulx 2005 |
| PTPN1                           | Tyrosine-protein phosphatase non-receptor type 1 | Tiludronate                      | 1                               | Inming et al., 2006 Freedman et al., 2001 Yin et al., 2017 | RJ He et al. 2014 Carty NC et al., 2012 |
| 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, Tetryahdrobioperin and L-arginine | 3                               | Marsden et al., 1993 Pilar et al., 2008 Shinkai et al., 2002 | Pitskars N. 2016 Lofiontiatis et al., 2016 Wass C et al., 2008 |
| CFTR                            | Cystic fibrosis transmembrane conductance regulator | Glyburide, Ivacaflo, Buprofen, Bumetanide | 4                               | Gillen et al., 2012 | Puljak Livia. 2006 |
| CASR                            | Calcium-sensing receptor     | Cinacalcet                       | 1                               | Hendy et al., 2013 Gupta et al., 2007 | BA Tugit et al. 2013 Kim JY et al., 2014 Dal Pra I et al., 2015 |
| CD3E                            | T-cell surface glycoprotein CD3 epsilon chain | Muromonab                       | 1                               | NA                               | FG Hoosian et al., 201 |

Table 2: Shortlisted druggable genes and their corresponding FDA approved molecules.
**Figure 1: Workflow: Multi-scale analysis of SZ genes**
Figure 2: Population distribution of personal, mutational range and common non-synonymous variants in SZ genes from non-psychiatric ExAC database. Amongst 4495 variants, 4045 mapping to 99 (out of 100 SZ genes) were identified in 6 populations. The variants observed in each population are directly proportional to their sample size. The bar diagram represents personal variants in blue color, the mutational range variants in red and the common variants in yellow color. AFR: African/African American; AMR: Latino; EAS: East Asian; FIN: Finnish; NFE: Non-Finnish European; SAS: South Asians.
Figure 3: 11 druggable candidate genes classified based on similarity in gene expression signatures. Blue: known drug targets for SZ; Red: putative drug targets for SZ. Amongst which SLC6A3, DRD2, DRD3, DAO, DAOA and CHRNA3 involve in neurotransmission.

Figure 4: Spatio-temporal expression profiles (Z_score RPKM) of druggable SZ candidate genes A.) DRD2, B.) DRD3 and C.) SLC6A3 in a developing human brain.