Spatio-Temporal Network Dynamics of Genes Underlying Schizophrenia

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Running title: A multi-scale analysis of Schizophrenia genes

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ABSTRACT

Schizophrenia (SZ) is a debilitating mental illness with multigenic etiology and high heritability. Despite extensive genetic studies the molecular etiology stays enigmatic. A systems biology study had suggested a protein-protein interaction (PPI) network for SZ with 504 novel PPIs amongst which several genes happen to be drug targets of existing FDA approved drugs. Although the PPI network presented all possible pairs of interactions (known and novel), it lacks a spatio-temporal information. The onset of psychiatric disorders is predominantly in adolescent and young adult stages, often accompanied by subtle structural abnormalities in multiple regions of the brain. Hence, there is a need to redefine the generic PPI network as a function of time (developmental stages) and space (brain regions). The availability of BrainSpan atlas data allowed us to redefine the SZ interactome as a function of space and time. The absence of non-synonymous variants in centenarians and non-psychiatric ExAC database allowed us to identify the variants of criticality. The expression of candidate genes in different brain regions and during developmental stages, responsible for cognitive processes as well as the onset of disease were studied. A subset of novel interactors detected in the network was further validated using gene-expression data of psychiatric postmortem brains. From the long list of drug targets proposed from the interactome study and based on the microarray gene-expression results, we have shortlisted a probable subset of 10 drug targets (targeted by 34 FDA approved drugs) coalescing into 81 biological pathways, that could be potentially repurposed for neuropsychiatric disorders.

Keywords: Schizophrenia, neuropsychiatric disorder, interactome, gene expression, BrainSpan, postmortem brain, pathways and drug targets.

INTRODUCTION

Neuropsychiatric disorders are progressively becoming important due to the increase in the disabling mental health conditions, social and economic consequences. SZ is a complex psychiatric disorder with multi-genic aetiology, affecting almost 1% of the global population (1). 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 (2). Complex neuropsychiatric diseases 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 (3). The National Human Genome Research Institute (NHGRI) of USA has catalogued 38 genome-wide association studies (GWAS) on SZ (4) revealing the association of common variants with SZ (5). The Psychiatric Genomics Consortium (PGC) has identified 108 SZ associated loci (6) which has revealed the role of common genetic variants in SZ. Moreover, the molecular mechanisms by which these genetic variations contribute to the syndrome of psychoses could be better understood by studying the continuum of 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 theoretically possible, though static, interactome for SZ. A few amongst them (pair-wise interactions) were experimentally validated (7). The analysis illustrates that despite the divergence between the genes or proteins found in various studies to characterize SZ, a commonality emerges through the interaction network, and the pathways, that the corresponding genes coalesce into. Several genes present in certain key pathways deduced from the interactome are already targets of drugs used to manage various chronic diseases (7).

Although thousands of 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 lacks a spatio-temporal information. SZ is, in essence, a developmental disorder and the onset is predominantly in adolescent and early adult stages (8). SZ is also strongly associated with subtle structural abnormalities and molecular differences in multiple regions of the brain (9). Hence, there is a need to redefine the network in specific developmental stages and brain regions, incorporating spatio-temporal information. The biological processes are dynamic, i.e. gene expression and molecular interactions differs over space (brain regions) and time (developmental stages). Though HiPPIP has led to a theoretically possible static interactome, the real biological networks are always small or a subset of the computationally predicted network. The genes must co-express and co-localize in-order to physically interact. Moreover, mRNA is less stable than the protein and the transcript abundance reduces due to mRNA degradation by ribonucleases

(10). The experimental evaluations were carried out in non-CNS tissues and it would be meaningful to validate the role of novel interactors in psychiatric postmortem brain tissues. Though the candidate genes used to construct the interactome were restricted to SZ GWAS findings, the corresponding genes and pathways have been strongly implicated with shared risk to other neuropsychiatric diseases like BP, ASD, MDD, OCD, addiction, substance abuse, etc. (11). Hence, all our investigations considered the complex genetic sharedness of various neuropsychiatric disorders and associated comorbidities (immune dysfunctions, metabolic syndromes, etc.), but not just restricted to SZ.

Antipsychotics (AP) have been in use since 1950s (12). 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, Olanzapine and Clozapine are commonly used and have been used to treat a range of psychotic illness, since their therapeutic effects are perhaps being mediated by dopaminergic and serotonergic receptor blocking activities (13). Antipsychotics have been associated with inducing long-term side effects such as weight gain (14), adverse metabolic effects, aggravating cognitive dysfunction (15) and many others. Lithium and Valproic acid have been administered to patients with Bipolar and Manic syndrome, but their mechanism of action is still relatively unknown (16).

The immense value of drug repurposing is well-understood today. Interestingly, 122 genes present in the SZ interactome are druggable, which in turn coalesce into 286 biological pathways (7). However, the expression profiles of those genes in normal and postmortem brain tissues, at various developmental stages, have not been investigated. Proteins must be present in spatio-temporal fashion in order to be a candidate for drug targets of repurposed drugs.

Hence, by integrating large data points from GWAS, non-psychiatric ExAC, Allen Brain Atlas, expression profiles of psychiatric postmortem brain samples, SZ interactome and gene-drug interaction network, we present a multi-scale analysis to improve our current understanding of the genomic complexity of neuropsychiatric disorders (4-7, 17, 18). The reason is that although several components of genomic variation are associated with the disease, their influence to the disease phenotype would necessarily have to act through

changes in the protein biology. Some of them would be: (i) Change in **activity/function** of protein (ii) Change in **quantity** of protein expression (iii) Change in **timing** of protein production and (iv) Change in **location** of protein production. Our "multi-scale" analysis addresses these 4 scales/factors 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 postmortem brain tissues), timing (multiple developmental stages) and location (multiple brain regions) of protein production, although the latter 3 is looked under the context of Ganapathiraju et al.'s network. Given the absence of the quantitative protein expression data, the gene expression (mRNA abundance) is correlated with the protein expression of SZ genes. Hence, the translational control and protein degradation pathways are not a part of the analysis. To do this, ExAC (non-psychiatric subset) data were mined into and the population distribution of rare and common non-synonymous variants in SZ genes were characterized to understand protein level changes. The lethal variants that were absent in centenarian genomes and non-psychiatric ExAC were also identified. 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 the critical genes and interactors as potential points of therapeutic interventions. We expect 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 also harnessed the transcriptomic data of psychiatric postmortem brain tissues from Gene Expression Omnibus (GEO) (18), to identify differentially expressed genes (DEGs) that overlap the genes present in SZ interactome. Some of the novel interactors showed differential expression in certain postmortem tissues and provided insights into psychiatric disorders and associated comorbidities like inflammation, immune dysfunction, visual deficits, etc. The druggable DEGs and the pathways they coalesce into were identified, presenting a probable subset of targets from the existing large gene-drug interactome (7) for repurposing existing drugs for psychiatric disorders.

RESULTS

Functional consequences of non-synonymous variants:

In order to characterise the functional implications of the non-synonymous variants in SZ candidate genes, we analyzed data from Ensembl Variation (EV) (19). 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 1) (20).

Identification and analysis 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, we identified those variants that were not observed in genomes of healthy centenarians (n=93) (21). The analysis revealed that 2004 (of 2037) lethal variants, which mapped to 99 genes, were absent in healthy centenarian genomes. Of the 2004, we found that 265 variants (mapping to 79 genes) were absent in non-psychiatric ExAC database, and were considered as variants of criticality. Further, In to include the variants that could turn deleterious later on under certain circumstances, we analyzed variants that were absent in centenarians but present in ExAC (non-psychiatric subset) database (17). On analysis, it was observed that 1739 lethal variants that mapped to 99 genes were present in non-psychiatric ExAC database, that were absent in centenarian genomes. These 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 commonly prevalent in populations. We limited our analysis to common and mutational range variants but not the personal variants since association of personal variants in complex disorders like SZ is minuscule. Hence, the 265 presumably critical variants might act 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 produce the disease phenotype. Hence, we present a panel of potentially deleterious 685 variants, that should be further investigated for behavioral phenotypes and brain patho-

biology in animal models of neuropsychiatric disorders (Figure 6) (Supplementary Table 1). It was 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 filtering process, the number of genes remain fairly the same. This could be because the risk variants are distributed among all genes identified by the GWAS and other association studies, which is also concordant with the existing polygenic theory that the risk for SZ is primarily driven by multiple genes with subtle effect sizes. Moreover, our assumption that some of the lethal and rare variants, that are absent in healthy centenarians, should map to the genes identified by GWAS (22), is primarily derived from the findings of Ganapthiraju et al., and also borne out by our results when we analyze the data.

In order to gain an overall snapshot of the genotype frequencies of variants present in SZ genes in global populations, we queried all 4495 variants present in non-psychiatric ExAC (without considering protein lethality or data from centenarian genomes). On analysis, it was observed that 4045 variants were mapped to 99 genes (out of 4495 variants in 100 genes). The AF of 4045 variants in 6 populations reported in the non-psychiatric ExAC database, were also retrieved (Supplementary Table 2). The analysis revealed that the variants observed in each of the population were directly proportional to their sample size. However, the proportion of the personal variants were higher (n=1843) in the out-bred European population (NFE) but were absent in the inbred Finnish (FIN) and East-Asian Tibeto-Burman (EAS) population (Figure 1). Although the personal variants were absent in FIN and EAS, the prevalence of psychiatric disorders was found to be high in both the populations (23). However, the absence of personal variants in FIN and EAS populations could be an artifact of the underpowered number of variations under investigation.

Finally, in order to verify the association of SZ candidate genes with other neuropsychiatric disorders we utilized OMIM and literatures which revealed 94 disease associated non-synonymous variants present in 37 SZ genes (Supplementary Table 3) (24). These variants were associated with other disorders include BP, MDD, Autism, Epilepsy and Seizure, Alzheimer's, etc. Amongst 94 variants only 22 were predicted to be lethal by PolyPhen

analysis which questions the real time accuracy of computational tools in predicting protein deleteriousness. Out of 22 so called lethal variants only 2 (rs3970559 and rs2904552) mapping to the same gene (*PRODH*) were absent in both centenarian and non-psychiatric ExAC data. Both the variants were strongly associated with cortical volumes in SZ. Although large GWAS and other association studies have pinpointed variants with odds ratio (OR) of strong statistical significance, we have verified their absence in the genomes of healthy centenarians in order to consider them as regions of criticality for the development of SZ.

Various studies have shown that most of the psychiatric disorders might be a consequence of the human brain evolution (25). In order to identify the genes that have undergone positive selection, we computed the pairwise dN/dS ratios of 100 SZ genes in Human-Chimp and Human-Dog lineages (Figure 2) (Supplementary Table 4). The mean dN/dS ratio of 100 genes in Human-Chimp lineage was found to be significantly higher than that of Human-Dog lineage (2 Sample t-test, P=2.03E-8). It was observed that only 2 genes i.e *MPHOSPH9* and *APOL2* were positively selected (dN/dS>1) in Human-Chimp and Human-Dog lineages, respectively. Previous studies have also revealed that *APOL2*, a gene responsible for lipid transportation and metabolism exhibited an increase in synonymous substitutions in chimpanzee as compared to dog. *APOL2* along with the Apolipoprotein-L genes which have undergone recent positive selection lies in 22q13.1 locus which is a hotspot for psychoses (26,27). The variant rs7285167 in the APOL2 gene was one amongst the 94 disease associated variants identified in our previous analysis although PolyPhen predicted it to be benign. *MPHOSPH9* also exhibited positive selection in Human-Chimp lineage, which was previously reported to harbor SNPs (Single Nucleotide Polymorphyims) that underwent recent selective sweeps in Asian populations (28). *TCF4*, *PPP3CC*, *DISC1, VRK2* and *SDCCAG8* showed less negative selection in human lineages. In order to identify human specific changes in the protein sequences of *APOL2* and *MPHOSPH9*, we carried out multiple sequence alignment of its corresponding protein sequences using Clustal Omega (29) (Supplementary Figure 2, 3). It was observed that 152 amino acids (AA) present in the N-terminal of *MPHOSPH9* protein sequence were present in humans but were absent in chimpanzee and gorilla (Supplementary Figure 2). Similarly, 112 AA present in N-terminal of *APOL2* protein sequence were present in humans and gorillas but were absent in chimpanzee (Supplementary Figure 3). Both the protein sequences were not annotated for any structurally

or functionally important protein domains in PROSITE database (30). Although human specific changes in the protein sequence in the above genes were observed, correlating the positive selection of *MPHOSPH9* and *APOL2* with the emergence of novel functions in human brain is difficult due to unavailability of structural annotation of the corresponding proteins.

Analysis of Spatio-Temporal Interactome:

Although the PPI map for SZ (7) presented all the possible pairs of interactions, we found that 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) (Figure 3) (Supplementary Table 5) (31,32). The top and bottom 10 nodes ranked by Hyperlink-Induced Topic Search (HITS) were considered as hub genes and non-hub genes respectively. With this study, we assumed that highly connected genes (hub genes) must be expressed significantly higher compared to non-hub genes in-order to interact with larger set of proteins and perform several functions. However, no difference was found between the gene expression means of hub genes and non-hub genes. In order to identify the genes that exhibit high variations in expression pattern in a normal human brain, we carried out median abolute deviation (MAD) score analysis for the 96 SZ candidate genes across its spatio-temporal gene expression data. The analysis revealed that the expressions of 24 genes were 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 tissues (Supplementary Table 6).

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 (33). According to our current understanding, most drug targets may not be ubiquitously expressed but enriched and localized in distinct tissues relevant to the disorders, even under normal conditions (34). Hence, there is a need to classify the drug targets from candidate genes based on their biochemical characteristics. 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 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 4). Amongst the sub-cluster of 11 genes, three (*SLC6A3, DRD2* and *DRD3*) have been well established drug targets for SZ (Figure 5) (7). *SLC6A3* was found to be active in child and adolescent thalamus, which has been implicated in SZ (Figure 5C) (35,36). *DRD2* (Figure 5A) and *DRD3* (Figure 5B) was found to be active only in Striatum across all developmental stages. Striatum has been well associated with the pathophysiology of SZ, BP, ASD and adolescent depression (37, 38, 39, 40, 41). *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 (42, 43). DAO is found to be enriched in anterior cingulate cortex (MFC) which has also been implicated in SZ (44). Interactors of *NAB2* are targeted by nervous system drugs to treat epilepsy, which often co-occur with SZ (7, 45). NAB2 is highly expressed in cerebellar cortex where SZ associated endophenotypes have been witnessed (46). Trace amine-associated receptors (*TAAR*) are GPCRs that are often activated after the blockade of dopaminergic receptors by antipsychotics (47). 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 to consider them for druggability. The spatio-temporal expression maps of 96 SZ genes are available in Supplementary File 1. Multiple evidences state that the therapeutic outcome of antipsychotics is mediated not just by the classical dopamine D2/D3 receptors, but also act indirectly with D1 receptors and probably several unknown targets. 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 (48). This multitude of effects and the latency of clinical outcome suggests a complex interplay of multiple unexplored biological pathways which needs to be understood using postmortem gene expression data for identifying relevant genes

in those pathways that could probably be repurposed as alternative drug targets for psychiatric disorders.

Analysis of differentially expressed genes in neuropsychiatric postmortem 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 postmortem brains were examined (18). Analysis of expression profiles of PFC, HPC and STR revealed 985 unique DEGs (FC>2; $P<0.01$) in nine different conditions (Supplementary File 2). The raw t-test *P*-values were used since FDR corrected *P*-values were not significant enough for most of the genes to be called differentially expressed. In order to validate the genes present in SZ interactome (especially novel interactors) (7) in postmortem brain tissues, we overlapped the gene IDs of 985 DEGs and 1718 genes in SZ interactome. 71 genes (2 candidate genes + 69 interactome genes) present in SZ interactome were differentially expressed in postmortem brain tissues, out of which 22 were novel interactors as predicted by Ganapathiraju *et al*., 2016. 14 of 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 literature evidences for psychoses associations. 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 2). Interestingly, 14 out of 71 DEGs are druggable targets of various FDA approved drugs according to the gene-drug interactome study (Supplementary Figure 4).

Analysis of druggable genes and pathways:

A 2-D matrix representing 286 biological pathways involving 122 druggable genes was constructed using the literature (7). From the above post mortem case control analysis, we identified 14 druggable DEGs. Of these 14 DEGs, 4 (*PTGS1*, *ERBB2*, *PTGER3* and *ESR2*) were found to be downregulated in either one amongst the nine conditions. Although the expression levels can vary, inhibition of highly expressed genes has been widely used since it is easier to block targets which are active (67). Therefore, the four downregulated targets were

excluded from downstream analysis, resulting 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 (Supplementary File 3) coalescing into 81 biological pathways (Supplementary Table 7).

Amongst 71 DEGs identified from postmortem brains, only 2 were candidate genes (*SLC6A4* and *CACNB2*) and 10 were druggable interactors. In order to identify more druggable genes present upstream or downstream in biological pathways involving the disease causing genes, a prior knowledge of the biological pathways that involves SZ candidate genes is required. This prompted us to utilize ConsensusPathDB (CPDB) (68) in-order to identify biological pathways in which 123 SZ candidate genes coalesce into (***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, G*α* signaling, Celecoxib pharmacodynamics, T-cell receptor signaling, Alzheimer's disease pathway, etc (Supplementary Table 8). Of these 54, only 3 had 4 putative drug targets (*COX-2*, *GNAQ*, *PLCB1* and *PDE10A*) which could perhaps be repurposed for psychiatric disorders. Their spatio-temporal expression (Z-score_RPKM) profiles were also provided (Supplementary Figure 5,6,7,8).

Analysis of biological pathways involving 4 putative drug targets:

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 used to treat several psychiatric disorders like MDD, BP and SZ (69, 70, 71, 72, 73). On analyzing the pathways over-representing 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 5).

G*α* signaling pathway:

The associations of G-protein coupled receptors and G*α* subunits in phosphoinositide signaling with psychoses has been well established (74,75). The two genes (*PLCB1* and *GNAQ*) were found to be involved in G*α* signaling pathway and deletions in the former were observed in SZ patients (76). The analysis revealed that the expression of *GNAQ* was high in Prenatal (CBC) (Supplementary Figure 6). However, expression of *PLCB1* was found to be high in V1C (Adult, Child and Infant) and Infant (STR) (Supplementary Figure 7).

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 which are currently being evaluated in clinical trials (77). 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 8).

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. With the advent of BrainSpan data, we have made an attempt to identify relevant candidate genes in the network which could influence the risk of psychoses. The non-synonymous variants for genes were picked up from 100 SZ candidate genes. 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. Population based studies are important to understand whether specific risk variants contribute similarly to the incidence of psychiatric disorders worldwide (78). We identified variants in SZ genes that are personal, common and in the mutational range by looking at population scale genomic data from non-psychiatric ExAC database, in 6 different populations. The expression profiles of 96 SZ candidate genes in a developing human brain were looked into, in 16 brain regions at 5

developmental stages and it was found that expression of 24 genes (20 genes to overlap with the final 88 SZ candidate genes), showed extreme variation. Thirteen of these 24 dynamic genes were found to be highly expressed in adult and adolescent brain regions, and might play a crucial role in the etiology of late onset psychiatric disorders like SZ, BP, MDD etc. 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 in a normal human brain and identified a sub-cluster of 11 genes (8 genes to overlap with the final 88 SZ candidate genes), that includes known drug targets *SLC6A3, DRD2* and *DRD3. DRD2*, the target of several antipsychotic drugs (37), is expressed predominantly in STR. Therefore, search for genes with similar expression profiles lead to the identification of 11 candidate genes that could be potential targets of novel 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 postmortem brains were looked into. Twenty two novel interactors present in the SZ interactome were found to be dysregulated in postmortem brains. These proteins previously had null or little associations with psychoses, thereby now validating a subset of the predicted interactors. We also report the dysregulation of *DHDDS***,** a gene thar has been strongly associated with Retinitis Pigmentosa, which occasionally co-occurs in certain schizophrenia cases (55). Although no direct evidence for psychoses was found for 8 novel interactors that were dysregulated in psychiatric postmortem brains, some of them (*MYOZ2, GSC2, MKI67* and *VANGL1*) were discernable and need further investigation. *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 (79,80). *GSC2*, a homeodomain containing gene resides on 22q11, which is a hotspot for psychoses (27). *MKI67* encodes a nuclear protein that is associated with cellular proliferation. SZ is often hypothesized to be a disorder of inappropriate neuronal proliferation and pruning (81), and hence role of *MKI67* in neuropsychiatric disorders could be further investigated. Mutations in *VANGL1* were associated with Neural-tube defects (82) which were also witnessed in SZ patients (83). From the long list of drug targets proposed from the interactome study, and based on microarray gene-expression results, we have shortlisted a probable subset of 10 druggable genes targeted by 34 FDA approved drugs, coalescing into 81 biological pathways, that could be potentially repurposed for neuropsychiatric disorders.

In conclusion, by leveraging publicly available multi-omics data we have performed a multi-scale analysis to improve the understanding of the genomic complexity of neuropsychiatric disorders. Although such approaches may yield insights using tissue level genomic data, deciphering the molecular interaction networks within terminally differentiated (TD) post-mitotic brain cells like neurons, etc., will be of criticality since SZ is also hypothesized to be a brain disconnection syndrome involving abnormal interactions between wide-spread brain networks (84,85). The identified druggable genes and biological pathways, though diverse, covers a broader spectrum of cellular functions such as survivability and proliferation, regulation of cell motility (mTOR), synaptic plasticity etc. Hence, validation of these pathways and genetic sub-networks in animal models of SZ, is of utmost importance to unravel the biology of mental illness, and also accelarating the drug repurposing pipelines.

METHODOLOGY

The entire analysis is represented as a graphical abstract (Figure 6)

Database mining of Single Nucleotide Variants of the Candidate Genes:

123 genes (101 interactome candidate genes + 22 OMIM genes) associated with SZ were retrieved from literature (7), which consists of both GWAS, Historic (pre-GWAS era) and OMIM genes set (Supplementary File 4). Their genomic co-ordinates (GRCh37) were extracted from Ensembl's Biomart (19). Well annotated non-synonymous variants were mined from Ensembl Variation (19) (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) (20). Polyphen predicts the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative evolutionary considerations. The lethal variants as predicted by PolyPhen were queried in (i) Centenarian database (21) and (ii) non psychiatric ExAC database (47,082 samples) (17). Non-psychiatric ExAC (version 0.3) variants with genotype quality≥20 and read depth≥10 were used for the above analysis. The dN and dS values were retrieved from BioMart interface of Ensembl Biomart. The genes and variants were then screened against several literatures, databases, including *Online Mendelian Inheritance in Man* (OMIM) (23) for disease associations.

Construction of Spatio-Temporal dynamic network:

The RNA-seq dataset from the BrainSpan Atlas of the Developing Human Brain (31), were retrieved for SZ candidate genes using the R package *ABAEnrichment* (31), 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 (Supplementary Table 5). The raw data was z-score normalized (gene-wise), and the Median Absolute Deviation (MAD) (scaling factor, k=1) in expression were calculated for each gene across all 16 tissues in 5 developmental stages. To facilitate 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 (number of connections it has to other nodes). The normalized spatio-temporal gene expression data was integrated into the interactome, with node size representing the expression levels of 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 in a normal human brain.

Postmortem microarray data:

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) (18). 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 were 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 were 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, were considered to be differentially expressed (Supplementary File 2). Annotation of Affymetrix probe IDs were perfomed 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 (7). The DEGs identified from the postmortem brain tissues were overlapped with 122 druggable genes, to identify the interactome drug targets that were differentially expressed in neuropsychiatric disorders. An independent analysis was carried out using ConsensusPathDB (Release 32) (68) to identify more druggable genes (apart from 122) in biological pathways in which SZ candidate genes coalesce into (**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.

Availability of data and materials:

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

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CONTRIBUTIONS

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 some of the results and editing the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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FIGURES

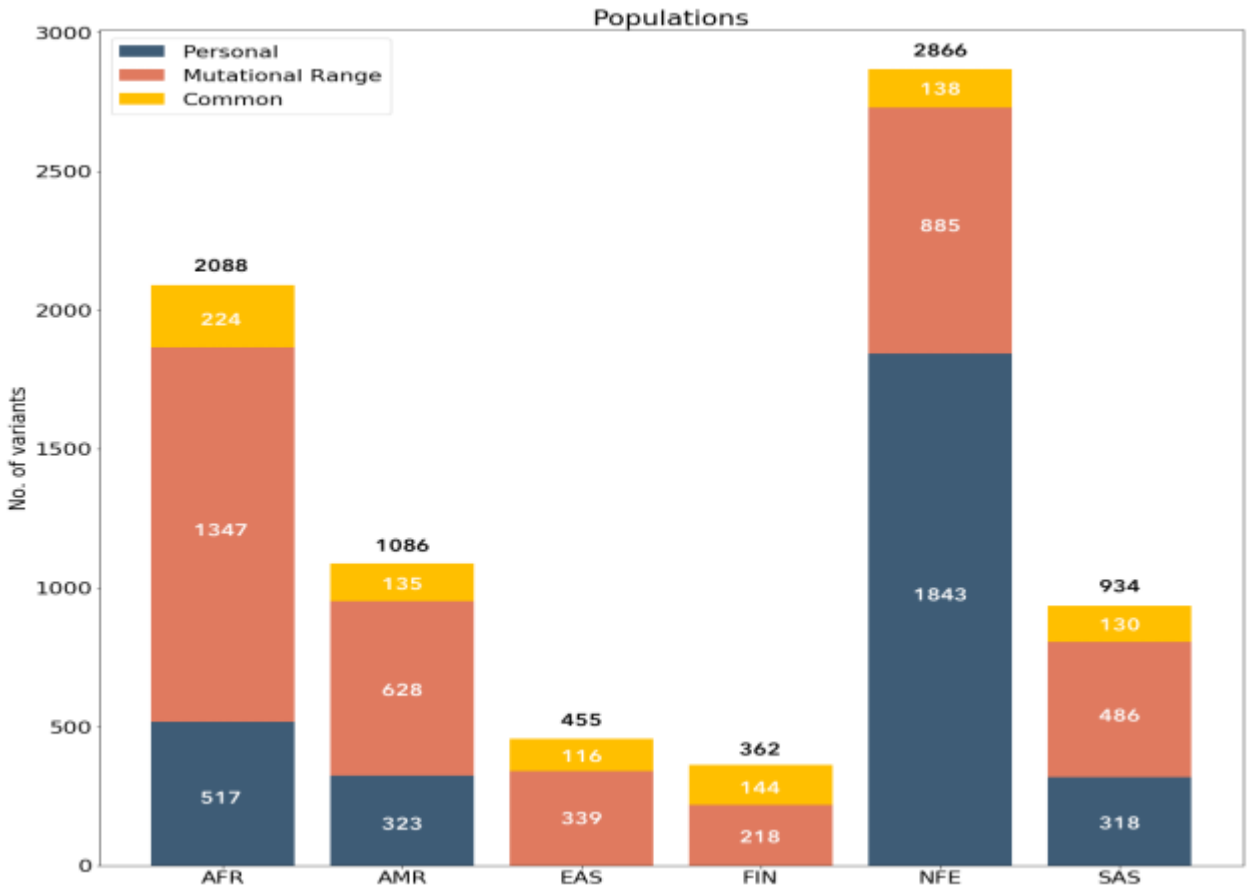

Figure 1: Population distribution of personal, mutational range and common non-synonymous variants in SZ genes from non-psychiatric ExAC database. Amongst 4495 variants, 4045 mapping to 99 (out of 100 SZ genes) were identified in 6 populations. The variants observed in each population are directly proportional 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.

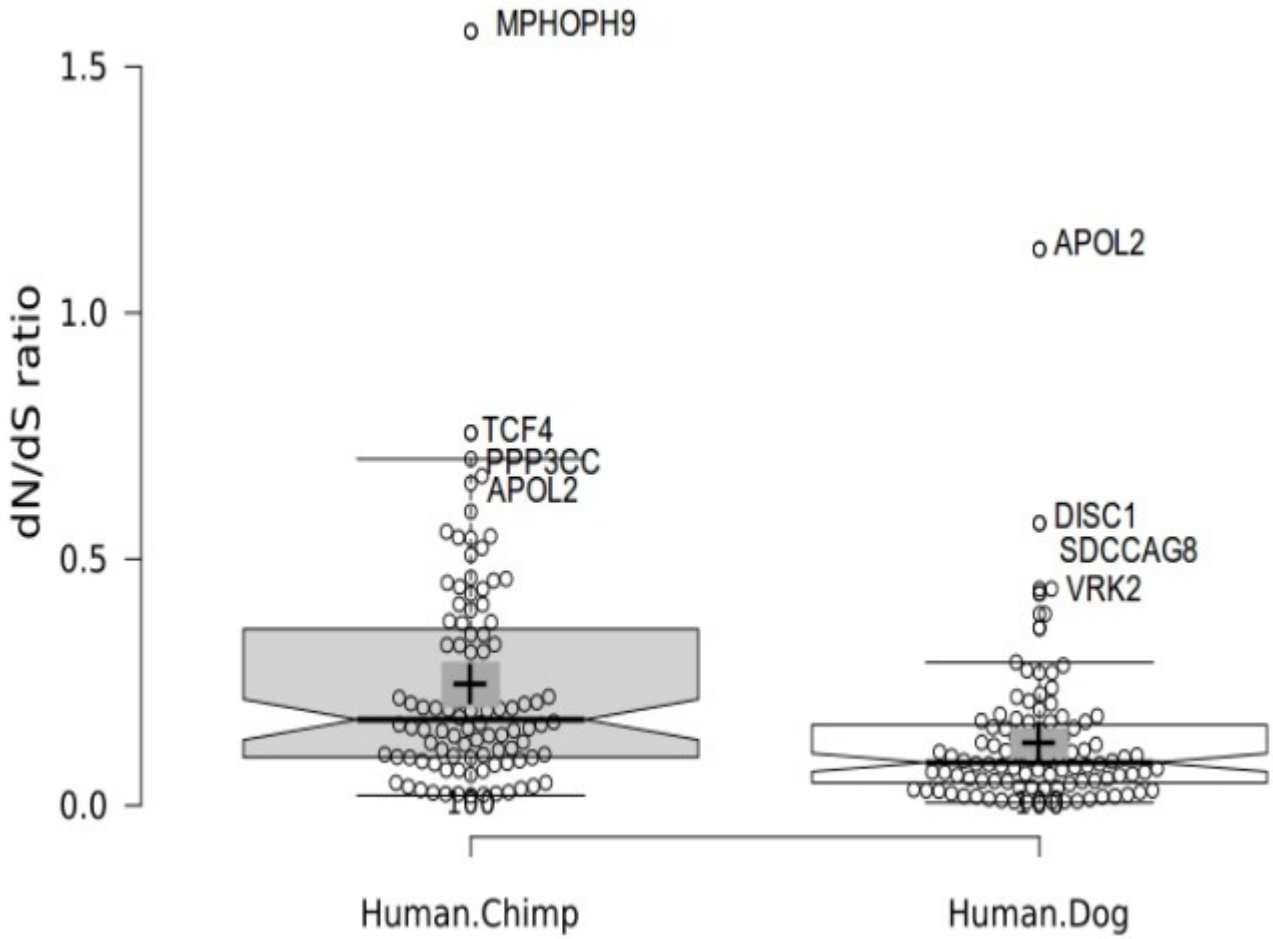

Figure 2: Ratio of non-synonymous to synonymous substitution rates in 100 SZ genes.Although significant increase in evolutionary selection was witnessed in Human-Chimp lineage compared to Human-Dog lineage (P=2.03E-8), most of the SZ GWAS loci are understrong negative selection.

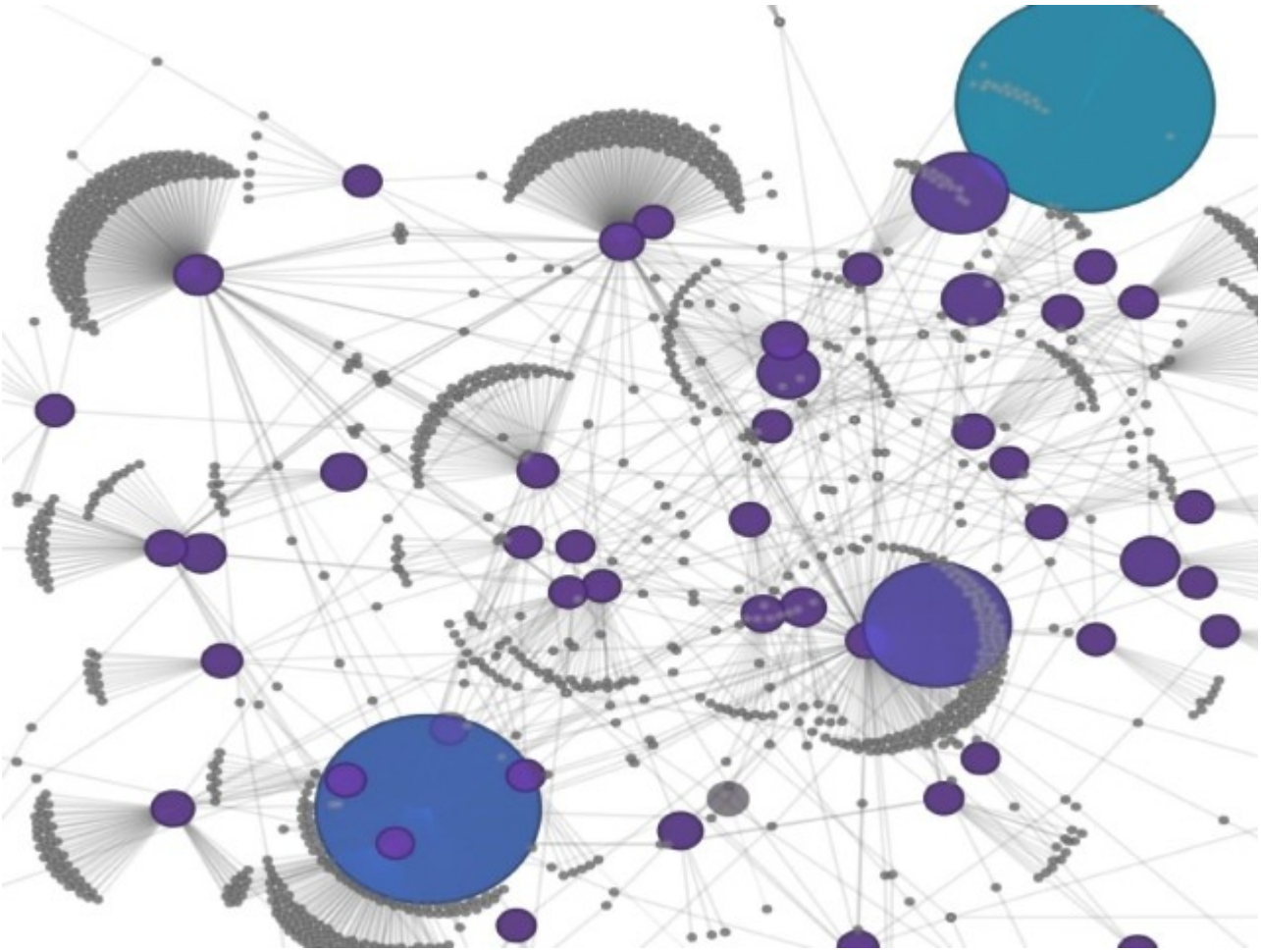

Figure 3: A snapshot of the spatio-temporal dynamic SZ interactome. Nodes representing the candidate genes are colored in violet (some nodes are set translucent to avoid obscurity of smaller nodes that may lie behind). The node size represents the expression of the corresponding gene which varies over different brain regions and developmental stages.

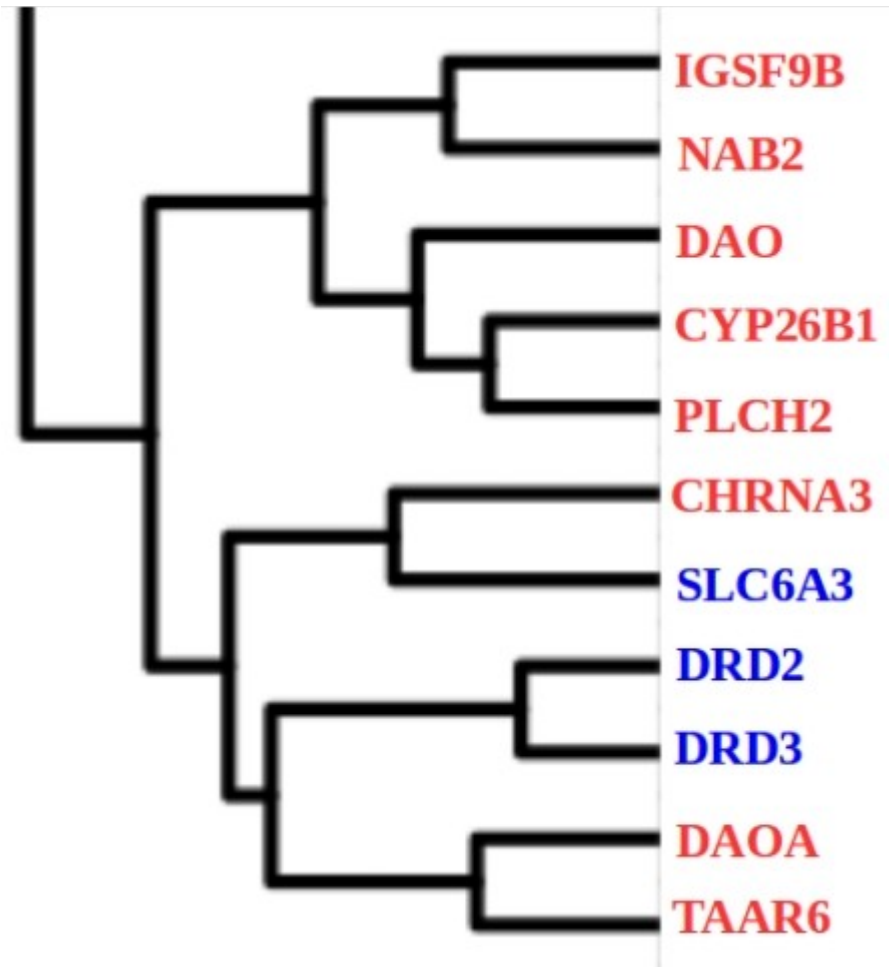

Figure 4: 11 druggable candidate genes classified based on similarity in gene expression signatures. Blue: known drug targets for SZ; Red: putative drug targets for SZ. Amongst which SLC6A3, DRD2, DRD3, DAO, DAOA and CHRNA3 involve in neurotransmission.

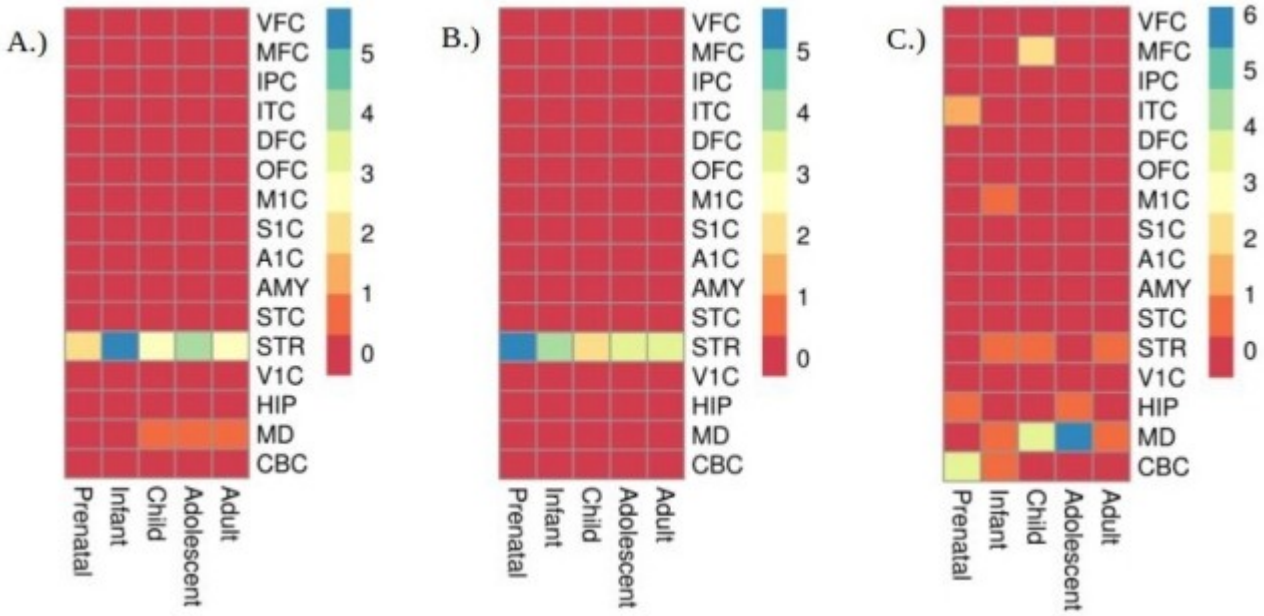

Figure 5: Spatio-temporal expression profiles (Z_score RPKM) of druggable SZ candidate genes A.) DRD2, B.) DRD3 and C.) SLC6A3 in a developing human brain.

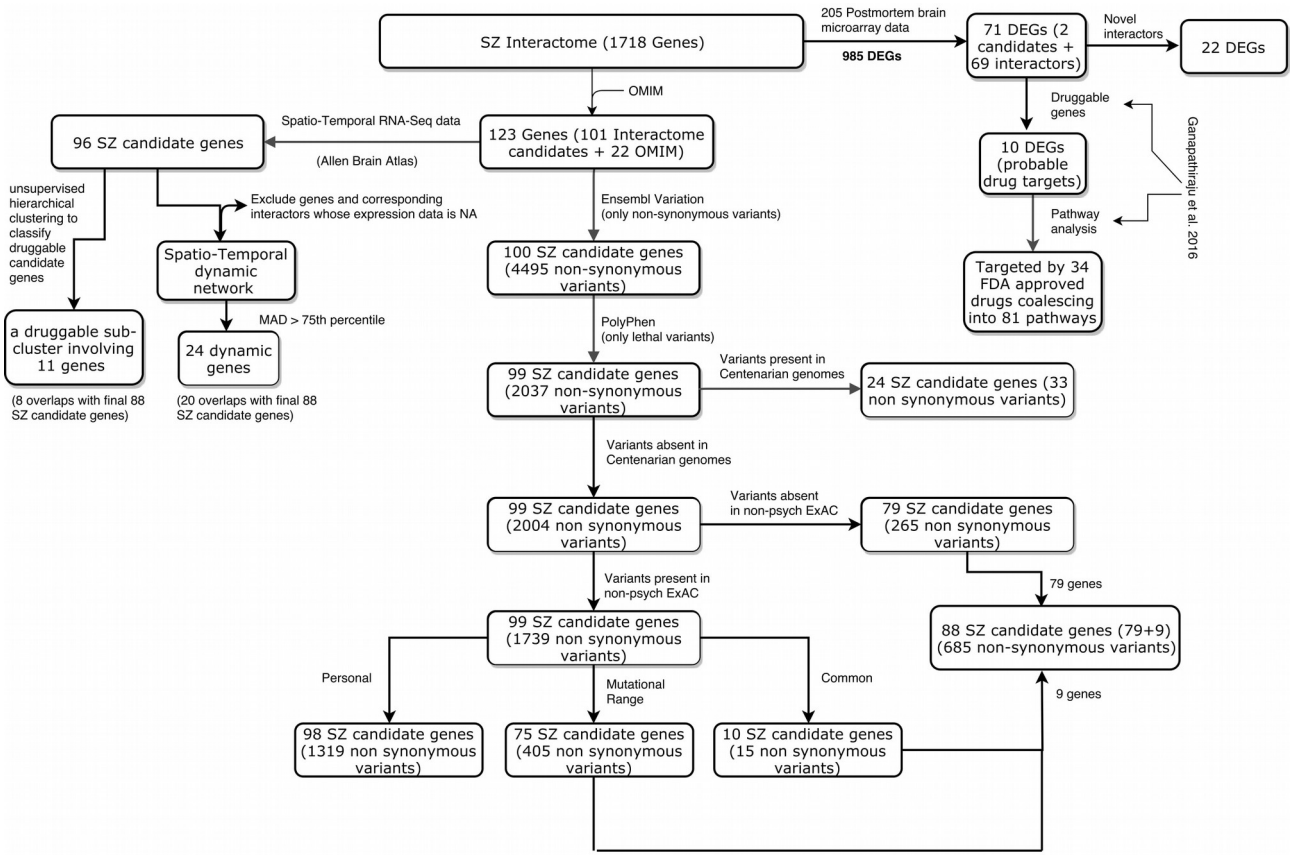

Figure 6: Workflow: Multi-scale analysis of SZ genes

TABLES

Gene_ID	Disorder (Tissue)	log_2FC	P-value	Previous evidence of association	References
PCDHGC5	SZ (PFC)	-1.09	0.004	Differentially expressed in differentiated neurons from clozapine responders	49
PDGFB	SZ (PFC)	1.16	0.001	*De novo* missense mutation associated with BD	50
	MDD (PFC)	1.08	0.004		
GNAS	SZ (HPC)	-1.65	0.003	Differentially methylated region (DMR) in monozygotic twins discordant for SZ	51
	MDD (HPC)	-1.17	0.004		
CD3E	SZ (HPC)	1.28	0.003	Associated with immunodefiency	52
	BP (HPC)	1.11	0.005	Polymorphisms associated with antidepressant response in Mexican-Americans with MDD	53
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	54, 55, 56
CD44	SZ (STR)	1.03	0.006	Upregulated in SZ postmortem DFC	57
ATP6V0A2	BP (PFC)	1.25	0.005	Mutations in same region repeatedly linked with BP	58
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.	59, 60
CD9	BP (PFC)	-1.19	0.004	Dysregulation observed along with myelination related genes	61
APOL1	BP (STR)	1.11	0.0003	SNPs found in strong haplotype in SZ affected families	62
CASR	BP (STR)	1.04	0.005	Upregulated in ischemic brain injury	63
AGF1G1	BP (STR)	1.11	0.008	Upregulated in SZ lymphoblastoid cell lines	64
PPP1R11	MDD (HPC)	-1.36	0.008	Resides within MHC class 1 loci, SZ hotspot	65
TACR3	MDD (HPC)	-1.61	0.001	Insignificant association for genotype/haplotype markers in Japanese populations	66

Table 1: Dysregulated novel interactors identified from postmortem microarray analysis.