Gene expression in patient-derived neural progenitors provide insights into neurodevelopmental aspects of schizophrenia

Oleg V Evgrafov[1,2,3*], Chris Armoskus[2], Bozena B Wrobel[4], Valeria N Spitsyna[1], Tade Souaiaia[1], Jennifer S. Herstein[1], Christopher P Walker[1], Joseph D Nguyen[1], Adrian Camarena[1], Jonathan R Weitz[1,5], Jae Mun 'Hugo' Kim[2], Edder Lopez Duarte[2], Kai Wang[1,2], George M Simpson[1], Janet L Sobell[1], Helena Medeiros[4], Michele T Pato[1,2,3], Carlos N Pato[1,2,3], James A Knowles[1,2,3]

[1]Department of Psychiatry and the Behavioral Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

[2]Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

[3]Current address: College of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA

[4]Caruso Department of Otolaryngology, Head and Neck Surgery, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

[5]Current address: Molecular Cellular and Developmental Biology program, University of Miami, Miami FL, USA

*Corresponding author

Conflict of interest.

The authors declare no conflict of interest.

Abstract

The PGC2 schizophrenia (SCZ) GWAS shows that variation in non-coding regions is responsible for most of the common variation heritability of the disease, suggesting risk variants alter gene expression. Therefore, comparing differences in gene expression between cases and controls may provide a direct approach for studying the etiology of SCZ. We studied transcriptome expression profiles of Cultured Neural progenitor cells derived from Olfactory Neuroepithelium lines (CNON) from 144 SCZ and 111 control individuals using RNA-Seq and identified 53 differentially expressed (DEX) genes (FDR<0.10).

Most DEX genes are implicated in Wnt or Notch signaling, or the metabolism of serine/glutamine/asparagine. DEX genes show enrichment for overlap with significant variants from the SCZ GWAS, and for GO terms related to neurodevelopment: cell differentiation, migration, neurogenesis, and synapse assembly. Our findings show the utility of gene expression analysis of neural progenitors for deciphering molecular aberrations associated with, and potentially causing, schizophrenia.

Introduction.

Schizophrenia (SCZ) is a devastating psychiatric disorder with estimated lifetime prevalence of 0.55% worldwide (1). With heritability of about 81% (2), genetics plays a critical role in the disease's etiology. Although causes of the disease remain a mystery, many direct and indirect pieces of evidences indicate that aberrations in brain development are major contributors (3–5). Post-mortem studies of the brains of individuals with SCZ provide important information about functional changes associated with the disease, but it is unlikely that the gene expression profiles of fully differentiated cells can identify all the genes responsible for aberrations in neurodevelopment. Even though the genetic mechanisms regulating neurodevelopment are poorly known, it seems reasonable to suggest that regulation of cell migration and the balance between cell division and differentiation of neuronal cells are critical

to proper brain development. Most neuronal cells in the adult brain are not dividing, migrating or differentiating, limiting the utility of adult tissue samples to find alterations in gene expression that affect neurodevelopment. In order to identify schizophrenia-related genes involved in neurodevelopment, we proposed to use *C*ultured primary *N*eural cells derived from *O*lfactory *N*euroepithelium (CNON) of individuals with, and without, schizophrenia (6). These cells are neural progenitors, actively divide, and migrate in 2D and 3D cultures. Here we present a study of transcriptome expression profiles using RNA-Seq (strand-specific, rRNA-depleted total RNA) in CNON lines derived from 144 SCZ and 111 control (CTL) individuals.

Results

We collected samples of olfactory neuroepithelium from the most superior posterior region of the nasal septum mucosa and from superior medial aspect of the middle turbinate and established CNON cell lines from 144 individuals with DSM-IV SCZ and 111 CTL (Supplementary Table 1), under approved IRB protocols (6,7). All CNON lines were cultured using the same lot of medium and cells were usually harvested after the third passage (see Online Methods). Strand-specific RNA-Seq of total RNA was performed after removal of cytoplasmic and mitochondrial rRNA, with an average of 23.38 million (7.1 – 106.7 million) 100 bp single-end reads that uniquely mapped to the transcriptome (Gencode 22) after exclusion of rRNA and mitochondrial genes (see Online Methods). DESeq2 was then used to normalize the read counts assigned to expressed genes and perform differential gene expression analysis. To facilitate the comparison of expression of each gene, normalized read counts were transformed to transcripts per million transcripts (TPM), using the union of all exons for each gene model as gene length (8).

Characterization of CNON cells.

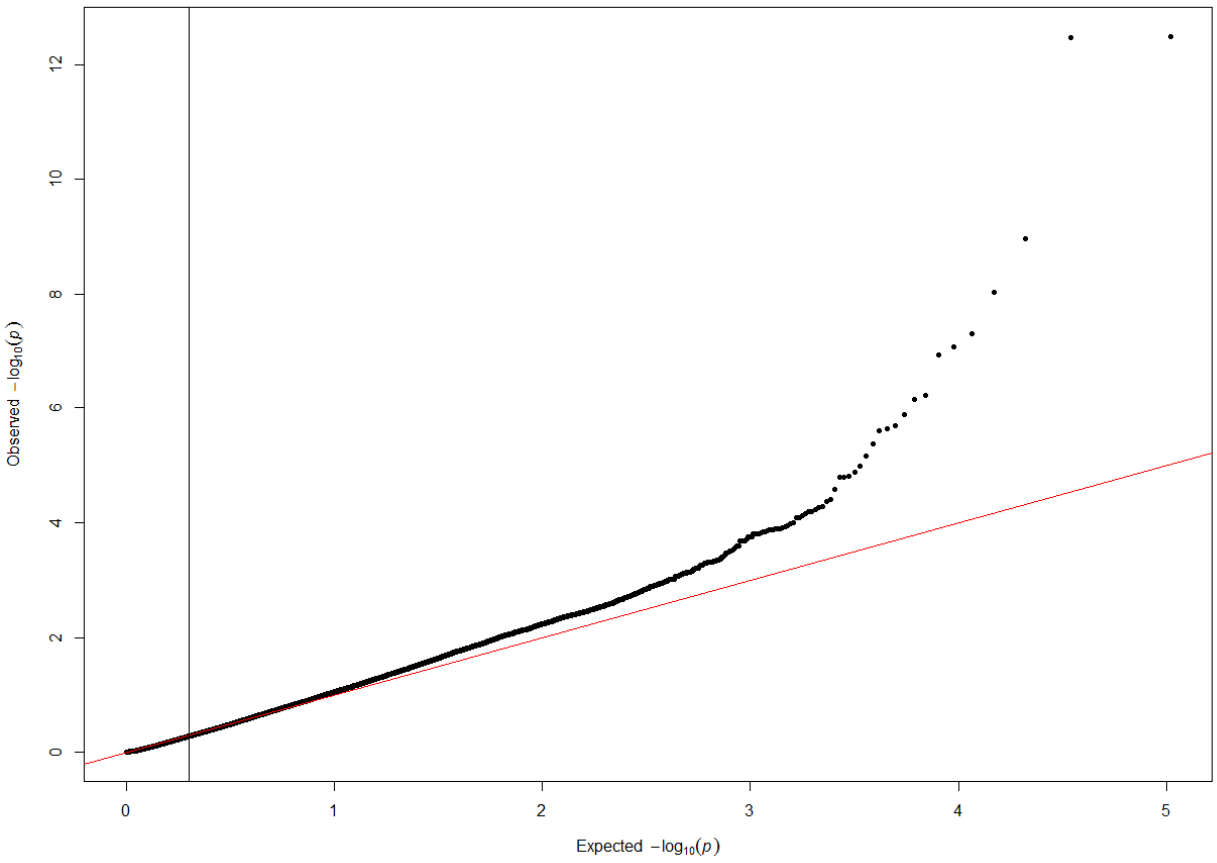
The RNA-Seq data in this study are consistent with our previous observations using Affymetrix Human Exon 1.0 ST arrays that CNON lines are neural progenitors (6) (see Supplementary Table 2 for more detail). In order to determine the point in human brain development the CNON lines most resemble, we compared RNA-Seq data from CNON to 647 post-mortem human brain samples across the lifespan (BrainSpan; www.BrainSpan.org). To mitigate the differences in RNA-Seq methodology (e.g., differences in read length and library construction), we transformed the gene expression values of each CNON sample using the coordinates described by the principle components of the BrainSpan data. The first principle component of the BrainSpan data roughly corresponds to developmental age and separates the pre- and post-natal samples (Supplementary Fig. 1). The CNON samples form a tight cluster within the prenatal samples, particularly those from the mid fetal period (weeks 13-24, roughly second trimester).

Genes differentially expressed in neural progenitor cells in SCZ

In order to build a statistical model for analysis of differentially expressed (DEX) genes, we examined multiple possible confounding factors and their contributions to variance, bias and inflation (see Online Methods and Supplementary Information for details). The final model includes covariates for sex, age, 2 principle components and one library batch, and results in a moderately conservative analysis (lambda = 0.88) (Fig. 1). Using this model, 53 genes (25 up in SCZ / 28 down) showed statistically significant differences between SCZ and CTL at an FDR of 0.1 (Table 1). Forty-three (81%) of the DEX genes are protein coding, and an additional 4 (8%) are processed pseudogenes with substantial expression (15 - 65 TPM). Thirty-two (60%) of the DEX genes are expressed at > 1 TPM. Seven DEX genes did not show a difference in median expression, or demonstrate a difference in median expression in the direction opposite to the

mean difference; this is a sign that the difference in mean expression between groups for these genes is caused mostly by outliers.

Figure 1. Quantile-quantile plot of SCZ vs. control differential gene expression p-values. Observed p-values from the analysis are matched to expected p-values based on quantiles of a uniform distribution (black). These points are compared to the line expected if the null hypothesis holds true for all genes (red line). The black vertical line marks the 50th percentile, where lambda is measured. Lambda, observed chi-square value with 1 d.f. vs. expected = 0.88, indicating slight deflation at this point.



Although we established that the overall effect of race/ethnicity on DEX analysis is negligible, we performed separate analysis taking into account race/ethnicity factor and identified 12 genes that fall out of significance after correction for race/ethnicity (Table 1). While differences between racial/ethnic groups in Principle Component Analysis (PCA) of genotypes

are considerable (Supplementary Fig. 2A), there is no similar separation of groups in PCA of gene expression (Supplementary Fig. 2B). Furthermore, adding covariates for race/ethnicity results in only small differences in significance (Supplementary Fig. 3). The most substantial effect of race/ethnicity on DEX status is seen in *HTR2B* gene, in which fold change among African-Americans is more pronounced than in other groups (Supplementary Fig. 4).

Table 1. Genes significantly DEX between SCZ and Control at FDR < 0.1. TPM is Transcripts Per Million, a measure of expression. Gene symbols are taken from Gencode release 25. Notes: 1) Fold-change in mean gene expression is reversed or close to zero in median gene expression, a sign that the difference in mean gene expression is tied to outliers; 2) Gene dropped out of significance when race/ethnicity was included in the model; 3) Gene lies under genome-wide significant GWAS peak; 4) De novo frameshift mutation in the gene has been identified in patient with SCZ; 5) De novo missense mutation(s) in the gene has been identified in patient with autism spectrum disorder; 6) CNVs were identified in multiple patients with SCZ

Ensembl ID	Gene Symbol	Gene Type	TPM	Log2 Fold-Change	FDR	Notes
ENSG00000149564.10	*ESAM*	Protein	0.527	-0.696	4.12E-09	1, 3, 4
ENSG00000157766.14	*ACAN*	Protein	0.367	-0.675	4.12E-09	
ENSG00000076706.13	*MCAM*	Protein	3.832	-0.577	8.46E-06	1, 5
ENSG00000144820.6	*ADGRG7*	Protein	0.41	0.462	5.50E-05	
ENSG00000170627.8	*GTSF1*	Protein	0.233	0.492	2.32E-04	
ENSG00000180447.6	*GAS1*	Protein	58.951	0.494	3.25E-04	
ENSG00000117115.11	*PADI2*	Protein	0.656	0.484	3.98E-04	
ENSG00000143631.10	*FLG*	Protein	0.307	-0.455	1.77E-03	5
ENSG00000115461.4	*IGFBP5*	Protein	719.099	0.475	1.82E-03	
ENSG00000117600.11	*PLPPR4*	Protein	0.858	-0.459	3.16E-03	
ENSG00000164532.10	*TBX20*	Protein	0.279	-0.421	4.47E-03	
ENSG00000203867.7	*RBM20*	Protein	0.275	-0.417	4.65E-03	
ENSG00000278099.1	*U1*	snRNA	11.724	0.387	4.67E-03	
ENSG00000174697.4	*LEP*	Protein	4.583	0.393	7.21E-03	
ENSG00000197461.12	*PDGFA*	Protein	3.097	-0.414	1.11E-02	1
ENSG00000128965.10	*CHAC1*	Protein	12.46	0.415	1.54E-02	
ENSG00000074181.7	*NOTCH3*	Protein	2.21	-0.407	1.86E-02	5
ENSG00000104722.12	*NEFM*	Protein	1.061	-0.41	1.92E-02	1
ENSG00000021645.16	*NRXN3*	Protein	0.314	-0.414	1.92E-02	

ENSG00000114251.12	*WNT5A*	Protein	77.485	0.356	1.92E-02	2
ENSG00000232184.1	*RP11-370K11.1*	Protein	2.367	-0.366	3.03E-02	3
ENSG00000170373.7	*CST1*	Protein	1.446	-0.333	4.23E-02	
ENSG00000163815.5	*CLEC3B*	Protein	10.387	0.389	4.45E-02	
ENSG00000169306.8	*IL1RAPL1*	Protein	0.179	-0.385	5.23E-02	6
ENSG00000073756.10	*PTGS2*	Protein	15.084	0.387	5.24E-02	
ENSG00000120324.6	*PCDHB10*	Protein	0.169	0.385	5.47E-02	
ENSG00000230787.1	*PSAT1P3*	Pseudogene	8.498	0.355	5.53E-02	
ENSG00000141469.15	*SLC14A1*	Protein	3.949	-0.339	5.53E-02	1
ENSG00000255583.2	*RP11-415I12.2*	Pseudogene	0.463	-0.381	5.63E-02	2
ENSG00000135069.12	*PSAT1*	Protein	48.816	0.352	6.11E-02	1
ENSG00000253810.1	*PSAT1P1*	Pseudogene	8.837	0.354	6.39E-02	
ENSG00000258071.1	*ARL2BPP2*	Pseudogene	6.12	0.253	7.38E-02	
ENSG00000182240.14	*BACE2*	Protein	18.203	0.262	7.68E-02	2
ENSG00000124107.5	*SLPI*	Protein	3.005	-0.247	7.92E-02	
ENSG00000226237.1	*GAS1RR*	lincRNA	1.74	0.278	8.12E-02	2
ENSG00000008517.15	*IL32*	Protein	1.425	0.367	8.12E-02	
ENSG00000112852.6	*PCDHB2*	Protein	0.404	0.354	8.12E-02	2
ENSG00000197181.10	*PIWIL2*	Protein	0.754	-0.365	8.12E-02	2
ENSG00000160801.12	*PTH1R*	Protein	0.208	-0.363	8.12E-02	2
ENSG00000111424.9	*VDR*	Protein	5.462	0.279	8.12E-02	2
ENSG00000070669.15	*ASNS*	Protein	19.656	0.312	8.33E-02	
ENSG00000135914.5	*HTR2B*	Protein	6.629	0.362	8.33E-02	2
ENSG00000154721.13	*JAM2*	Protein	5.253	0.36	8.33E-02	2
ENSG00000179136.5	*LINC00670*	lincRNA	2.323	-0.305	8.33E-02	
ENSG00000184564.8	*SLITRK6*	Protein	0.502	0.361	8.33E-02	2
ENSG00000179772.7	*FOXS1*	Protein	1.141	-0.262	8.90E-02	
ENSG00000223361.5	*FTH1P10*	Pseudogene	1.41	-0.345	8.90E-02	
ENSG00000254827.4	*SLC22A18AS*	Protein	0.455	-0.359	8.90E-02	
ENSG00000145936.7	*KCNMB1*	Protein	0.324	-0.341	9.30E-02	1
ENSG00000131620.16	*ANO1*	Protein	0.479	-0.347	9.41E-02	2
ENSG00000189058.7	*APOD*	Protein	4.089	0.356	9.41E-02	
ENSG00000232111.1	*RP11-126O22.1*	Pseudogene	0.195	0.356	9.41E-02	
ENSG00000105281.11	*SLC1A5*	Protein	136.402	0.214	9.41E-02	

To validate our DEX gene list we performed a comparison to gene expression values determined using RT-qPCR. Expression of 5 genes (selected from DEX genes in tested subset of samples including genes with fold change differences in both directions and spanning a range of gene expression levels) plus *ACTB* (used for normalization) was measured in 146 samples. Expression data from RT-qPCR and RNA-Seq across 146 samples were highly correlated within each gene (mean r = 0.74, range 0.61 - 0.84, all p-values < $2x10^{-12}$) (Supplementary Fig. 5A), and mean expression of the six genes had correlation r = 0.949 between two approaches (p = 0.0038) (Supplementary Fig. 5B). DEX of 4 of 5 genes was replicated, while one gene (*PLAT*) did not reach significance (p = 0.15), but shows a trend in the same direction (Supplementary Fig. 6).

Permutation analysis of differential expression

To assess the probability that our DEX findings could be due to random statistical variation, we performed two forms of permutation analysis. For the first, we randomly permuted the diagnosis labels but held all other factors constant and found a median of 13 DEX genes at FDR < 0.1 (mean = 36.9). We used the Wilcoxon signed rank test to assess significance of the mean signed rank between permuted and experimental data, and the number of DEX genes found in permutation analysis is significantly lower (p = $8.1x10^{-8}$) than in the original analysis. This shows the difference in gene expression between the actual CTL and SCZ groups is greater than that between random groupings.

For the second permutation analysis, we compared groupings where the null hypothesis should hold (SCZ-SCZ and CTL-CTL), to those where we expect the null hypothesis to be violated (SCZ-CTL; two different sample sizes matching SCZ-SCZ and CTL-CTL comparisons). The null comparisons resulted in far fewer DEX genes at FDR < 0.1 (SCZ-SCZ, median=9.2, mean=11.7; CTL-CTL median=2.0, mean=20) as compared to SCZ-CTL (medians = 19 and 16 DEX genes, means = 91.1 and 191, for two sample sizes, respectively). Most DEX genes (e.g., *WNT5A*, *GAS1*, *PSAT1*) were never significant in SCZ-SCZ or CTL-CTL comparisons, whereas

a few genes, such as *NOTCH3* and *ESAM,* were significant in a few comparisons (4 of the 120, and 8 of the 120 null comparisons, respectively), consistent with their higher observed heterogeneity of expression, tied to more outliers. However, it should be noted that *ESAM* had the highest "true positive" rate based on significance in random subsets comparisons of SCZ-CTL and the lowest (most-significant) median FDR of 0.007. These two analyses indicate that our results, based on our specific analysis model, are unlikely to be due to chance, but rather are identifying genes with actual differences in mean expression between the SCZ and CTL populations.

Power Analysis

Power in an RNA-Seq experiment depends on a number of variables: fold change and depth of sequencing for a given gene, the biological coefficient of variation (BCV), and the significance threshold/alpha (9). Given the nature of an FDR, the more genes that are found significant, the higher the effective alpha and power are. The median fold change for genes found DEX at FDR < 0.1 in our experiment is 1.29 ranging from 1.16 to 1.62. The median BCV of expressed genes (depth >= 3.5) is 0.414, which is in line with other reports for variation among humans between the same type of cells (9). The median depth of sequencing of DEX genes was 30.6.

Given a BCV of 0.414, if we look at power with our sample size based on a threshold of FDR < 0.1 with 50 significant DEX genes, power at the median fold change observed (1.29) is 23.4% at a depth of 3.5, increasing to 77.3% at a depth of 30. The expected power at the minimum to maximum fold change seen ranges from 2.4% to 97.3% at the minimum depth, or from 13.3% to 99.9% at the median depth (Supplementary Fig. 7A). However, the biological coefficient of variation is significantly larger in DEX genes than it is in other expressed genes, even after removing effects associated with SCZ (Mann-Whitney test p-value < $2x10^{-16}$) with a median of 1.03. If this BCV is more characteristic of genes affected by SCZ, then power

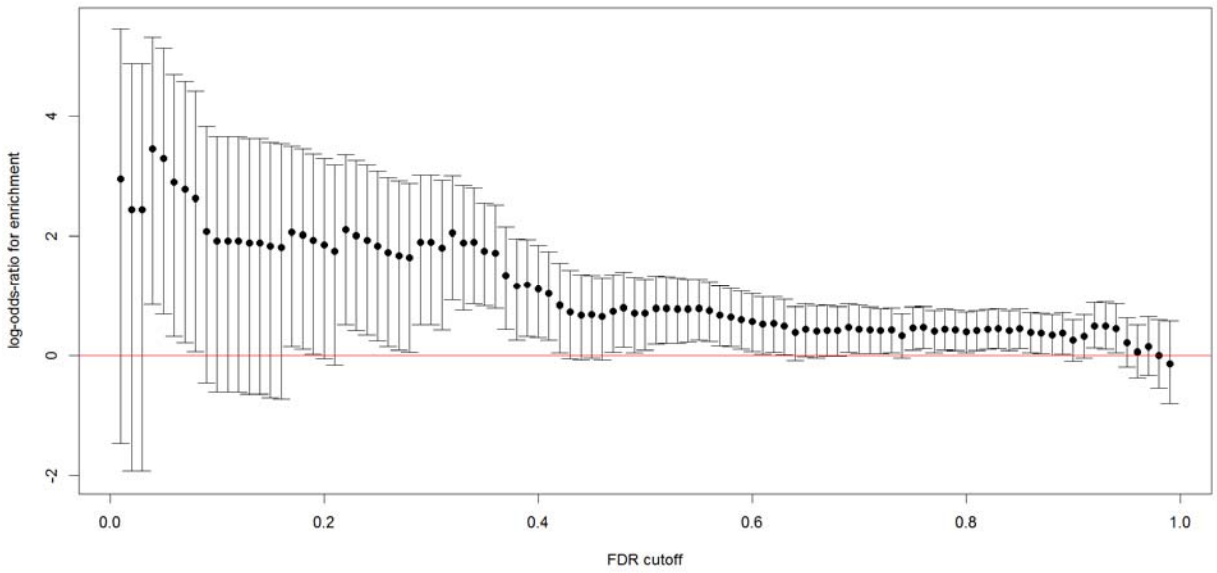
estimates for detecting such genes are substantially lower, only 3.8% at the median fold change and median depth (range 0.5% to 48.1% for minimum and maximum fold changes) (Supplementary Fig. 7B).

GWAS enrichment analysis

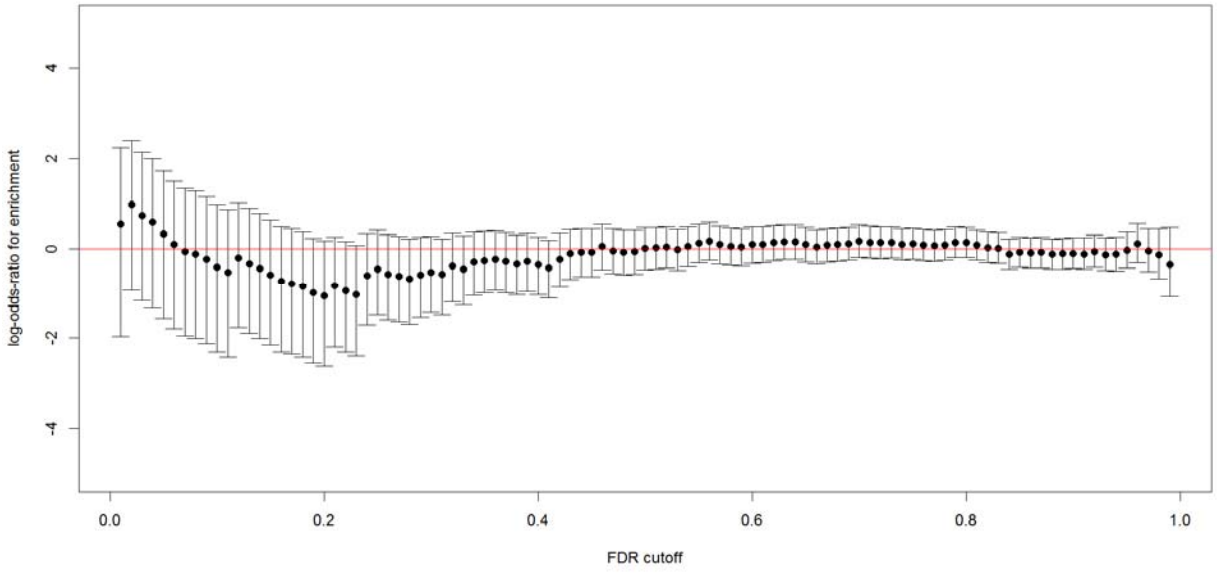
We tested the hypothesis that DEX genes are more likely to be co-localized with genome-wide significant ($p < 5x10^{-8}$) signals from the PGC2 SCZ GWAS (10), excluding the HLA region of chromosome 6. Two DEX genes (FDR < 0.1), *ESAM* and *RP11-370K11.1* (located within an intron of *AKT3*), were co-localized with genome-wide significant GWAS variants. Inspection of the PGC2 GWAS and our DEX list reveals multiple genes that just miss being significant in one analysis or the other. A variant within *IL1RAPL1*, another DEX gene, almost reached GWAS significance ($p = 5.75x10^{-8}$); *ITIH3* and *FOXO3*, containing genome-wide significant variants, are #67 and #93 in the list of the most significant DEX genes, and *GABBR2* is close to significance both in GWAS ($5.48x10^{-7}$) and DEX (#62) (Supplementary Table 3). Given the relatively low power of the study and conservative DEX analysis, we expect that most of the true DEX genes have not reach significance in the analysis at the FDR < 0.1 threshold. Relaxing the FDR threshold and including more potentially true positive findings may improve the power to detect enrichment. Analysis of the full range of FDR values (from 1% to 99%) showed significant enrichment of genes co-localized with genome-wide significant SCZ GWAS results at 69 of the 99 test points (Fig. 2, panel A). The odds-ratio generally decreased from its peak at OR = 10.96 at FDR of 4% down to a plateau of around OR = 1.35 from 0.6 to 0.8 and remains above 1 at all tested points except when using a 99% FDR threshold. Peak significance for enrichment was found at an FDR of 35% (317 genes), with 10 genes (*ESAM, RP11-370K11.1, ITIH3, FOXO3, SRPK2, TMEM110-MUSTN1, AC073043.1, CDC25C, MPHOSPH9, ALG1L13P*) being co-localized with GWAS-significant SNPs (p = 0.001; OR = 3.4).

Figure 2. Plot of log2-odds-ratio for enrichment of DEX genes, based on FDR cutoff values ranging from 1% to 99%, co-localized with GWAS-significant variants (95% confidence interval shown). Panel A shows DEX genes between SCZ and control groups, while panel B and panel C show similar data for DEX genes by sex and age, respectively. Values of negative or positive infinity are censored, as seen in the negative infinities (no co-localized DEX genes) for low FDR values and genes DEX by age.

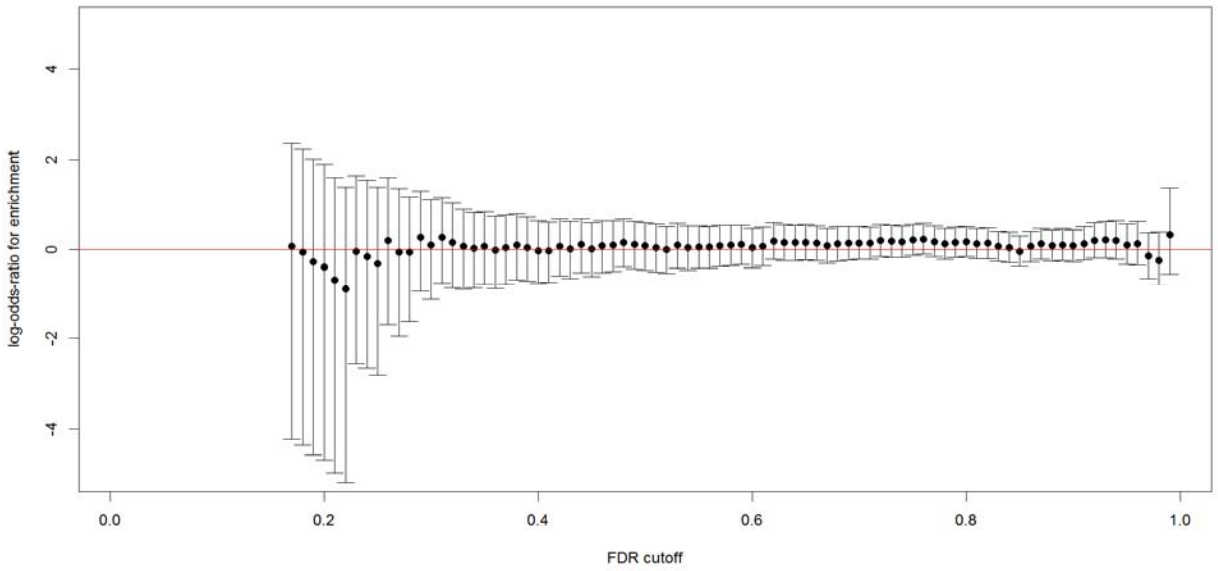
A



B



C



For comparison, we also tested for enrichment of co-localization with PGC SCZ2 GWAS signals for genes DEX by sex or by age. Neither analysis showed significant enrichment at any FDR threshold (Fig. 2, panels B and C). P-values for enrichment in these 99*2=198 comparisons were all greater than 0.13. Odds ratios were seen both above and below 1 even at low FDR thresholds, in contrast to SCZ DEX gene enrichment. These analyses support our finding of enrichment of SCZ DEX genes co-localized with GWAS peaks.

Gene set enrichment, pathway and network analyses of DEX genes

Gene set enrichment analysis (GSEA) of the DEX genes (FDR < 0.1) found that 32 out of 53 genes are annotated with the GO term “single-organism developmental process” (corrected p = 2.07×10^{-6}), an expected finding for neurodevelopmental aspects of SCZ (Table 2, Supplementary Table 4). Other development-related GO terms such as “cell differentiation”, “cell motility”, “cell migration” and “neurogenesis” also reached significance. Although CNON cells do not form synapses, the list of significantly enriched GO terms also includes “synapse assembly” and “synapse organization”.

Table 2. Gene Ontology enrichment analysis results based on 53 DEX genes (FDR < 0.1). Analysis done by g:Profiler using the ordered query option that takes into account which genes are more significant. Hierarchical filtering was applied to produce only the most significant term per parent term. Complete table of significant terms is presented in Supplemental Table 4. P-values were corrected for multiple comparisons by algorithm g:SCS, the default option in g:Profiler.

GO Term Name	GO Term ID	Corrected p-value	DEX Genes
Single-organism developmental process	GO:0044767	2.07e-06	32 genes
System development	GO:0048731	7.49e-06	26 genes
Synapse assembly	GO:0007416	6.51e-04	6 genes: *IL1RAPL1, NRXN3, PCDHB2, PCDHB10, SLITRK6, WNT5A*
Lipoprotein lipid oxidation	GO:0034439	1.37e-02	2 genes: *APOD*, *LEP*
Regulation of lipid biosynthetic process	GO:0046890	1.46e-02	5 genes: *HTR2B*, *LEP*, *PDGFA*, *PTGS2*, VDR
Locomotion	GO:0040011	1.74e-02	10 genes
Cell migration	GO:0016477	1.88e-02	9 genes: *ESAM*, *IGFBP5*, *LEP*, *MCAM*, *PADI2*, *PDGFA*, *PTGS2*, *TBX20*, *WNT5A*

Analysis of the 53 DEX genes with Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com) found that the function “differentiation of cells” was significantly enriched ($p = 2.72x10^{-3}$), and had the highest activation z-score of 2.359. The activation score assesses the direction of gene regulation together with the direction of the effect and predicts increased cell differentiation in SCZ, as compared to CTLs.

Similar to analysis of enrichment of DEX genes in GWAS-associated loci, GSEA may benefit from relaxing threshold p-value for DEX genes, and “ordered query” option further reduces penalty for increasing the list of DEX genes. Thus, we also analyzed expanded group of 317 genes with FDR-corrected $p < 0.35$ (where the most significant overlap with GWAS signals was recorded). The significance of aforementioned enriched GO terms in this expanded DEX gene set increased (Supplementary Table 4), indicating presence of additional genes associated with the same GO terms and further supporting involvement of corresponding

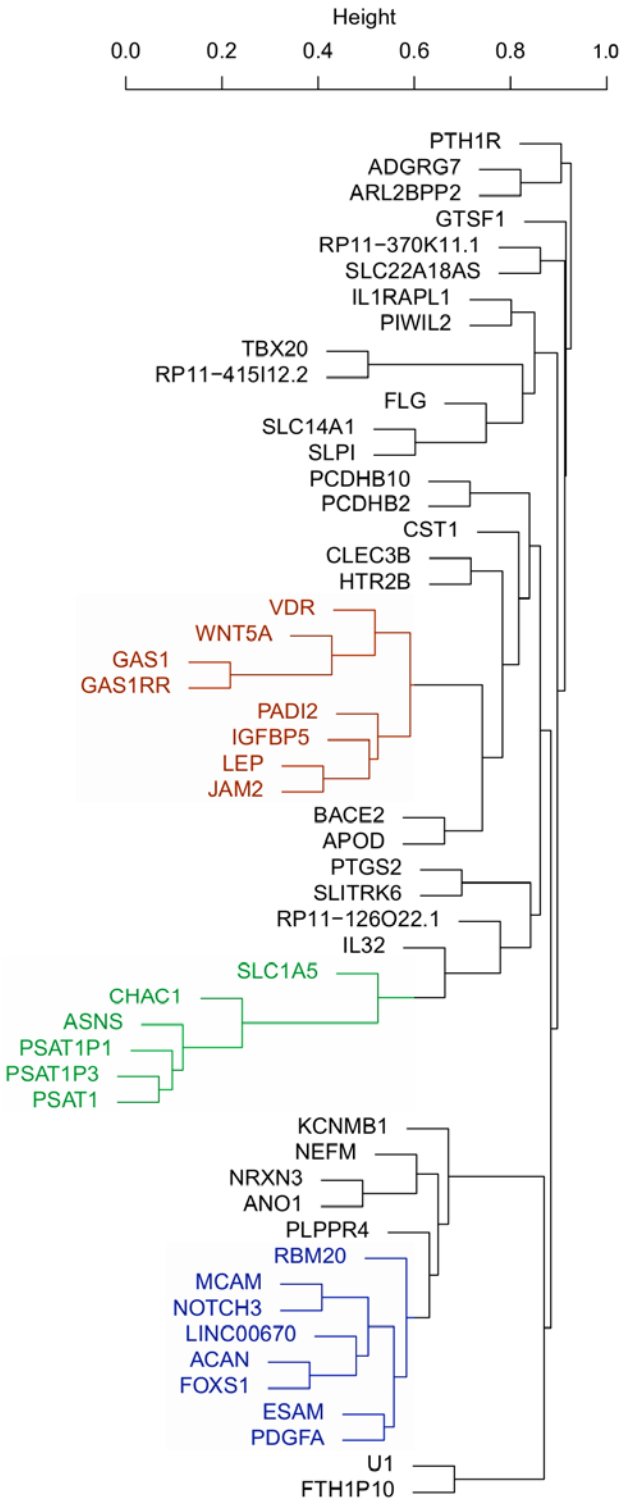
biological processes in the etiology of SCZ. Additionally, multiple GO terms related to cell proliferation become significant.

We performed similar GSEA analysis of genes (except not using ordered query) with non-silent *de novo* mutations found in patients with SCZ, which we compiled from the article of Fromer et al. (11). Mutations cover all primary genetic causes of SCZ, similar to GWAS, while our gene expression profiling is capable of identifying only genes whose expression is aberrant in SCZ in neural progenitor cells, thus mostly defining neurodevelopmental aspects of the disease. However, genes with mutations in SCZ show more significant enrichment in multiple GO terms related to neurodevelopment than those which are related to synapse development and function (Supplementary Table 5). Notably, the same terms “single-organism developmental process”, “cell differentiation”, and “neurogenesis” are significant in both expression profile and mutation GSEA. This suggests overall importance of neurodevelopmental aberrations in etiology of SCZ, and demonstrates general agreement between two genetic approaches.

Hierarchical clustering of DEX genes

Analysis of correlation between expression of just the DEX genes is an alternative method to find relationships within this group of genes. Hierarchical clustering based on this characteristic shows three distinct clusters of correlated genes (Fig. 3), which collectively include almost half of DEX genes. Every one of these groups contains functionally related genes.

Figure 3. **Hierarchical clustering of DEX genes.** Clustering was performed using average linkage and a distance of one minus the absolute value of the correlation. Sub-clusters containing more than 2 genes with a distance of less than 0.6 between them (absolute value of correlation > 0.4) are emphasized by color. These sub-clusters are composed of genes that all vary in the same direction.



Two of these groups are related to Wnt and Notch signaling. The third group includes genes related to amino acid synthesis/transportation and consists of *PSAT1*, its pseudogenes *PSAT1P1* and *PSAT1P3*, *ASNS*, *CHAC1* and *SLC1A5*. Although *CHAC1* is known to inhibit Notch signaling through deglycination regulating neurogenesis (12), and could functionally link two of the clusters, expression of this gene is not correlated with *NOTCH3* in our sample. Ingenuity Pathway Analysis did not find any enrichment in canonical pathways (after correction for multiple comparisons), but identified *WNT3A* as a top upstream regulator of DEX genes (p-value of overlap 1.24×10^{-9}).

Discussion

The choice of an appropriate cellular model is critical for gene expression studies of human diseases. To study SCZ-specific aberrations in gene expression affecting neurodevelopment, we chose to use cultured neural cell lines developed from olfactory neuroepithelium (CNON), and demonstrated that they are neural progenitor cells based on their expression profile (6). Neural progenitor cells divide, migrate and differentiate into glia and neurons, thus being critical for defining the composition and structure of the brain. Moreover, the expression pattern of CNON cells is most similar to second trimester fetal brain (BrainSpan, www.brainspan.org), a critical period for development of SCZ, affirming that CNON is a relevant model to study neurodevelopmental aspects of the disease.

With expected subtle differences due to SCZ and relatively high effects from confounding factors, it is critical to appropriately design experiments to minimize batch effects, identify and correct data for different sources of bias and use controls to ensure adequacy of correction procedures. Our reasons for choosing this statistical model, and the evidence that our correction procedure is sufficient to remove most biases and identify genes relevant to SCZ, are described in detail in the Supplementary Material. Importantly, we control for inflation based on the QQ-plot (Fig. 1) and lambda, assuring that the analysis is not inflated. Our model indicates

some deflation (lambda = 0.88), thus being slightly conservative according to this criterion. We provide strong support that this data analysis was proper based on the results of two types of permutation analysis, investigation of diagnostic plots and, most importantly, study of biological relevance of results. Using this analysis of the total long RNA-Seq data of CNON lines from 144 SCZ and 111 CTL individuals, we identified 53 differentially expressed (DEX) genes at FDR<0.1.

Currently, the most robust information about the genetic basis of SCZ comes from the large-scale SCZ GWAS (PGC2 SCZ) (10), which identified 108 LD-independent genomic regions containing variants reaching genome-wide significance. Most of the associated genomic regions are intergenic or intronic, suggesting they increase the risk of SCZ by altering the function of regulatory regions, such as promoters and (more probably) enhancers (13). Thus, it seems reasonable that gene expression studies should correlate with GWAS. However, regulation of gene expression in cells is much more complex than simple interaction between one associated genomic variant and one gene; other regulatory elements and expression of many other genes, such as miRNA, long non-coding RNA, transcription factors, other protein-coding genes, are involved in direct or indirect gene expression regulation. It is the integration of all these factors which ultimately defines gene expression profile and, correspondingly, cellular characteristics. Thus, given the complexity of relationship between genotypes and expression profiles, and low power of both GWAS and DEX studies, we should expect only moderate enrichment of GWAS signals in DEX genes. The results fit our expectations, showing modest, but still substantial and statistically significant overlap. Genome-wide significant variants are located within two DEX genes, *ESAM* and non-coding *RP11-370K11.1* (located within intron of *AKT3*, a proposed candidate gene for SCZ (14)), and *IL1RAPL1* contains a SNP that is nearly genome-wide significant (rs5943629, $p = 5.748 \times 10^{-8}$).

As shown in Table 1, many DEX genes have support from multiple types of genetic studies, such as GWAS (*ESAM, RP11-370K11.1, IL1RAPL1*), copy number variations

(*IL1RAPL1)* (15)*, de novo* mutation analysis in individuals with SCZ (*ESAM*) (16) or with autism spectrum disorder (*MCAM, NOTCH3, FLG*) (17). *IL1RAPL1* also has mutations in individuals with mental retardation (18) and autism (19). Taken together, these data demonstrate convergence of different genetic approaches.

Comparison of our results with those from a study of SCZ DEX genes performed on post-mortem dorsolateral prefrontal cortex (20) found only one gene in common (*CLEC3B*), with expression changing in the opposite direction. The lack of overlap may be due to differences in the biological material (neural progenitor cells vs. adult brain tissue) or in the statistical analysis (e.g., the accepted amount of inflation (Supplementary Fig. 8)).

More than half of the DEX genes are related to three pathways: Wnt, Notch and serine biosynthesis. This relationship becomes apparent from hierarchical clustering analysis of the DEX genes, revealing three clusters of co-expressed genes, supported by known functional relationships between genes in each cluster. Additionally, some DEX genes outside these clusters are functionally related with one of these pathways.

All DEX genes related to Wnt signaling change their expression in a direction corresponding to increased signaling in SCZ. *WNT5A* (p-value rank #20), which encodes a Wnt ligand, is over-expressed. Wnt ligands are known to induce expression of *GAS1* (#6) (21), encoding a pleiotropic regulator of cellular functions (22), which is also over-expressed. Among the functions of GAS1 is attenuation of SHH signaling (21), another key pathway in neurodevelopment. In particular, *GAS1* regulates proliferation of the external germinal layer and Bergmann glia thus defining the size of the cerebellum (23), one of the most prominent characteristics affected in SCZ (24). *PLPPR4* (#10) encodes a member of the lipid phosphate phosphatase family that is specifically expressed in neural tissue and antagonizes canonical Wnt signaling (25), and is under-expressed. In addition, *VDR* (#38), whose product potentiates Wnt signaling during development (26), is also over-expressed. *FOXO3* (#95, Supplementary Table 3) modulates Wnt signaling and is located under a GWAS-significant peak (10). Other top

genes have also been linked to Wnt signaling: *IGFBP5* (#9) (27), *LEP* (#14) (28) and *JAK1* (#54) (29). Analysis of top upstream regulators of DEX genes by IPA also supports the conclusion that altered (enhanced) Wnt signaling is a common feature of SCZ neural progenitors.

Numerous studies based on both genetic and functional data implicate a key role of Wnt signaling in neurodevelopmental aberrations in SCZ (reviewed in (30–32)). Moreover, the transcriptome study of Topol et al (2015) (33), performed on neural progenitor cells developed from human iPSCs, identified the Wnt pathway as the most altered between SCZ and CTL cell lines, with the same direction of aberration as in our study. Although only 4 SCZ and 6 CTL human iPSCs-derived cell lines were analyzed, the fact that the same pathway was found altered in both studies supports the idea that it could be one of the common pathways directly or indirectly affected in SCZ.

Another cluster of DEX genes is related to Notch signaling. This molecular pathway regulates neural progenitor maintenance and differentiation (34), is involved in cell migration (35) and, together with Wnt signaling, plays a pivotal role in differentiation (36). Levels of the expression of most of the key Notch signaling genes (*NOTCH2*, presenilins, *ADAM10*, *ADAM17, RBPJ, JAG1*), do not differ between SCZ and CTL. However, *NOTCH3* and multiple genes involved in regulation of, or regulated by, Notch signaling are different in their mean expression between SCZ and CTL groups. Moreover, expression of every one of these genes differs in SCZ in the direction corresponding to reduction of Notch signaling. For example, *NOTCH3*, a Notch receptor associated with neural differentiation (37), is under-expressed in SCZ, while *CHAC1* (and *JAK1* ranked #54, just outside of significance), whose products repress Notch signaling, are over-expressed. *NEFM*, one of the markers of neuronal differentiation, is under-expressed, and *APOD*, downregulated by NOTCH3 (38), is over-expressed. Several other DEX genes are also related to Notch signaling: *PTH1R* (protein product of this gene

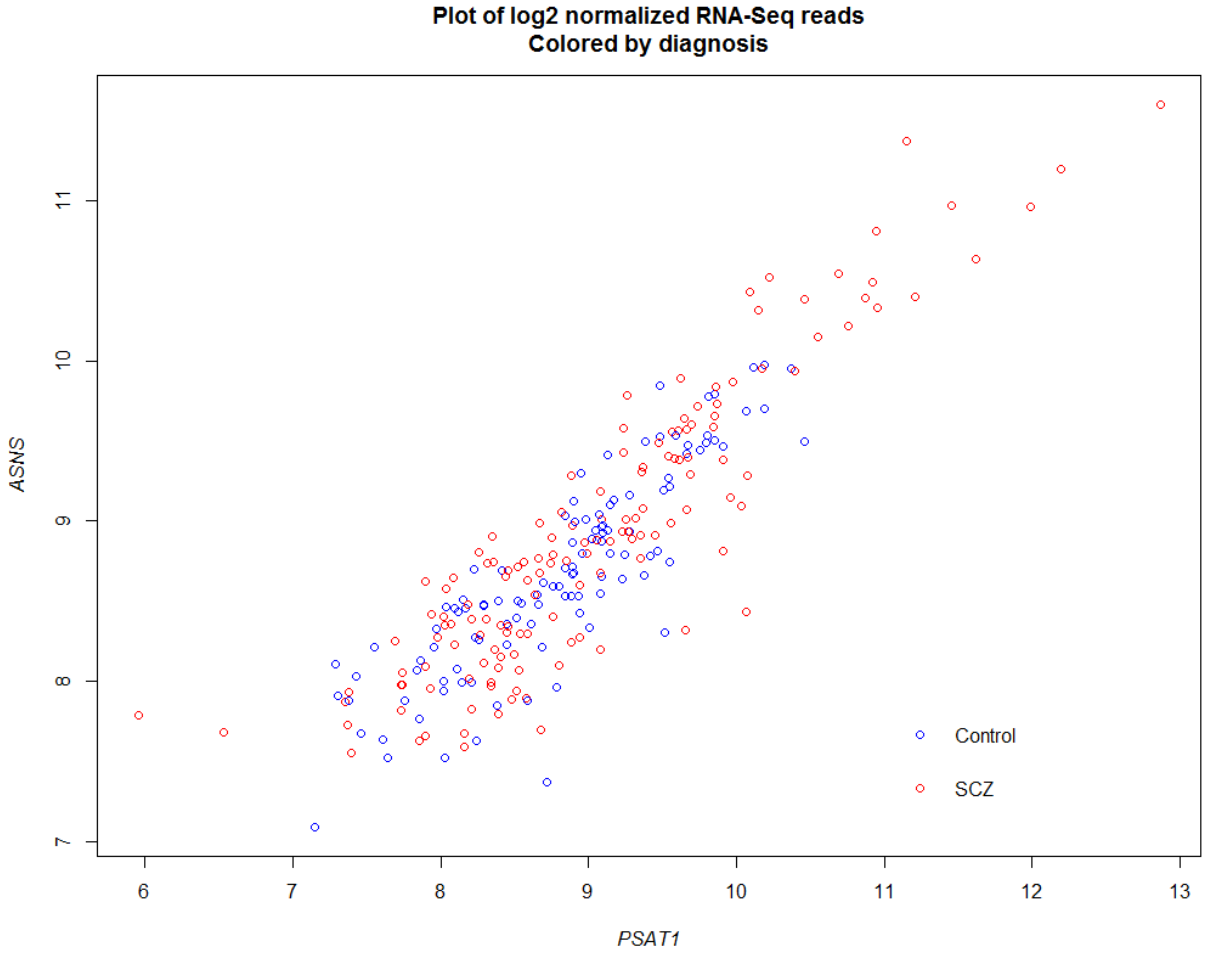
stimulates expression of Jagged1, ligand of Notch receptors), *TBX20* (corresponding protein is known to specify sensory organ within Notch signaling pathway).

NOTCH3 expression correlates with a group of cell adhesion genes (*ESAM*, *ACAN* and *MCAM*), the most significantly differentially expressed and with the largest fold changes, that are under-expressed in SCZ. Notch signaling controls differentiation of dendritic cells in spleen, which results in cells expressing *ESAM* at either a high or low level (39), suggesting a causal relationship between expression of *NOTCH3* and this cell adhesion gene. It should be noted that substantial contribution to the mean difference between SCZ and CTL groups in expression of genes correlated with *NOTCH3* comes from CTL samples showing increased expression (Supplementary Fig. 9).

While involvement of Wnt and Notch pathways in developmental aspects of SCZ is expected if not warranted based on the role of these pathways in neurodevelopment, discovery of DEX genes involved in serine/asparagine biosynthesis (*PSAT1, PSAT1P1, PSAT1P3, ASNS, CHAC1, SLC1A5*) is somewhat surprising. A substantial subset of SCZ patients can be separated from the CTL group and other affected individuals based on expression of these genes (Fig. 4). Comparing enzymatic reactions of highly co-expressed DEX genes *PSAT1, ASNS* and *CHAC1*, we notice that all three of them are directly (*PSAT1, ASNS*) or indirectly (*CHAC1*) involved in consumption or production of glutamate. Both *PSAT1* and *ASNS* are involved in biosynthesis of amino acids, serine and asparagine respectively, and *SLC1A5* is a neutral amino acid transporter, known to transport serine, glutamine and possibly asparagine. High correlation between expression of *ASNS* and genes involved in biosynthesis of serine is not specific only for CNON cells (40), and may indicate functionally justified tight coordination of expression of these genes. Asparagine is used as an amino acid exchange factor and regulates uptake of serine, which, in turn, attenuates serine biosynthesis (40). Regulation of uptake of serine and some other amino acids may play a role in coordinating of mTORC1 activity and, consequently, protein and nucleotide syntheses as well as cell proliferation (40). The finding that

asparagine synthesis is essential for the development and function of the brain but not for other organs (41) indicates that disturbance of this biological function of asparagine could result in alteration of brain structure underlying SCZ.

Figure 4. Scatterplot of *PSAT1* and *ASNS* gene expression. Expression of the genes shows high Pearson correlation (r = 0.895). A subgroup with high expression of both genes is composed solely of SCZ samples.



Mutations in *PSAT1* affect neurodevelopment (42), and the gene is implicated in SCZ based on a study of gene expression in a family with chromosomal translocation near *PSAT1* associated with SCZ and schizotypal personality disorder (43). D-serine regulates proliferation and neuronal differentiation of neural stem cells (44) involved in hippocampal neurogenesis (45). It is also a potent agonist of the N-methyl-D-aspartate receptor (NMDAR), and may play a

role in the brain beyond neurodevelopment. The level of this amino acid is on average decreased in SCZ (46), and it is used for SCZ treatment. It is worth mentioning that *DAOA*, a prominent SCZ candidate gene, encodes an activator of D-amino acid oxidase involved in degradation of D-serine. Additionally, aspartate, D-serine and L-glutamate serve as neurotransmitters. Another gene from the serine biosynthesis pathway, *PSPH,* shows the same tendency to be over-expressed in SCZ, being nominally significant. Moreover, all three genes in this pathway (*PSAT1, PSPH* and *PHGDH*) demonstrate both highest and lowest expression level in SCZ samples (Supplementary Fig. 10), suggesting that abnormal serine intracellular concentration, regardless of direction of change, may predispose to or be a part of molecular pathophysiology of SCZ. WGCNA analysis found a gene module related to amino acid biosynthesis that is enriched with DEX genes (Fisher's exact test $p = 8.2x10^{-7}$) and includes *PSAT1, PHGDH, ASNS* and *CHAC1*. The eigengene for this module also shows differential expression between SCZ and CTL groups (p=0.04), supporting our findings of alterations in serine biosynthesis in the disease.

There are also excellent SCZ candidate genes among DEX genes outside these three pathways. *HTR2B* encodes a serotonin receptor; this class of molecules is a long-time suspect as a major player in etiology of schizophrenia (47). Lately, a hypothesis of involvement specifically *HTR2B* in SCZ etiology gained support (48,49). *PCDHB2* and *PCDHB10,* together with other protocadherins, are involved in cell migration and communication and participate in synapse assembly. Thus, most DEX genes identified in this study are functionally related to neurodevelopment and indicate particular pathways and signaling systems which may be affected in SCZ.

In summary, DEX analysis of neural progenitor cells derived from olfactory neuroepithelium in SCZ and CTL showed pronounced differences in expression of genes related to Wnt and Notch signaling, and serine/asparagine biosynthesis pathways. DEX genes are enriched in genes involved in biological processes related to neurodevelopment, and overall

are good candidate genes to explain aberrations in brain development. DEX genes have significant overlap with SCZ-associated GWAS loci, and indicate aberrations in the same pathways or biological processes as study of *de novo* mutations in psychiatric disorders and expression profiling of neural progenitor cells generated from patient-derived iPSCs. Thus, our results are consistent with the latest genetic studies of SCZ performed by three different approaches, further supporting CNON as a powerful and efficient model for studying neurodevelopmental mechanisms of the disease.

Online Methods

Methods, including detailed explanation and justification of data analysis, are available in the online version of the paper.

Competing Financial Interest

The authors declare no competing financial interests

1. Goldner EM, Hsu L, Waraich P, Somers JM. Prevalence and incidence studies of schizophrenic disorders: a systematic review of the literature. Can J Psychiatry [Internet]. 2002 Nov [cited 2015 May 30];47(9):833–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12500753
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a Complex Trait. Arch Gen Psychiatry. 2003;60:1187–92.
3. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry. 1987;44(7):660–9.
4. Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. Annu Rev Neurosci [Internet]. 2002 Jan [cited 2015 Mar 19];25:409–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12052915
5. Raedler TJ, Knabel MB, Weinberger DR. Schizophrenia as a developmental disorder of the cerebral cortex. Curr Opin Neurobiol. 1998;8:157–61.
6. Evgrafov O V, Wrobel BB, Kang X, Simpson G, Malaspina D, Knowles J a. Olfactory neuroepithelium-derived neural progenitor cells as a model system for investigating the molecular mechanisms of neuropsychiatric disorders. Psychiatr Genet [Internet]. 2011 Oct [cited 2011 Dec 20];21(5):217–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21451437

7. Wrobel BB, Mazza JM, Evgrafov O V., Knowles JA. Assessing the efficacy of endoscopic office olfactory biopsy sites to produce neural progenitor cell cultures for the study of neuropsychiatric disorders. Int Forum Allergy Rhinol. 2013;3(2):133–8.

8. Wagner GP, Kin K, Lynch VJ. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. Theory Biosci. 2012;131(4):281–5.

9. Hart SN, Therneau TM, Zhang Y, Poland GA, Kocher J-P. Calculating sample size estimates for RNA sequencing data. J Comput Biol [Internet]. 2013 Dec [cited 2016 Jul 4];20(12):970–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23961961

10. Ripke S, Neale BM, Corvin A, Walters JTR, Farh K-H, Holmans P a., et al. Biological insights from 108 schizophrenia-associated genetic loci. Nature [Internet]. 2014;511:421–7. Available from: http://www.nature.com/doifinder/10.1038/nature13595

11. Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, et al. De novo mutations in schizophrenia implicate synaptic networks. Nature [Internet]. Nature Publishing Group; 2014;506(7487):179–84. Available from: http://www.nature.com.myaccess.library.utoronto.ca/nature/journal/v506/n7487/full/nature12929.html

12. Chi Z, Byrne ST, Dolinko A, Harraz MM, Kim MS, Umanah G, et al. Botch Is a□??-Glutamyl Cyclotransferase that Deglycinates and Antagonizes Notch. Cell Rep [Internet]. The Authors; 2014;7(3):681–8. Available from: http://dx.doi.org/10.1016/j.celrep.2014.03.048

13. Gusev A, Lee SH, Trynka G, Finucane H, Vilhjálmsson BJ, Xu H, et al. Partitioning Heritability of Regulatory and Cell-Type-Specific Variants across 11 Common Diseases. Am J Hum Genet [Internet]. 2014 Nov 6 [cited 2014 Nov 6];95(5):535–52. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4225595&tool=pmcentrez&rendertype=abstract

14. Howell KR, Floyd K, Law AJ. PKBγ/AKT3 loss-of-function causes learning and memory deficits and deregulation of AKT/mTORC2 signaling: Relevance for schizophrenia. PLoS One. 2017;12(5):e0175993.

15. Melhem N, Middleton F, McFadden K, Klei L, Faraone S V, Vinogradov S, et al. Copy number variants for schizophrenia and related psychotic disorders in Oceanic Palau: risk and transmission in extended pedigrees. Biol Psychiatry [Internet]. 2011 Dec 15 [cited 2016 Feb 21];70(12):1115–21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3224197&tool=pmcentrez&rendertype=abstract

16. Xu B, Ionita-Laza I, Roos JL, Boone B, Woodrick S, Sun Y, et al. De novo gene mutations highlight patterns of genetic and neural complexity in schizophrenia. Nat Genet [Internet]. Nature Publishing Group; 2012;44(October):1–7. Available from: http://dx.doi.org/10.1038/ng.2446

17. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature. Nature Publishing Group; 2012;485(7397):246–50.

18. Carrié A, Jun L, Bienvenu T, Vinet MC, McDonell N, Couvert P, et al. A new member of the IL-1 receptor family highly expressed in hippocampus and involved in X-linked mental retardation. Nat Genet [Internet]. 1999 Sep [cited 2016 Mar 19];23(1):25–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10471494

19. Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, Mottron L, et al. Mutations in the calcium-related gene IL1RAPL1 are associated with autism. Hum Mol Genet [Internet]. 2008 Dec 15 [cited 2016 Mar 19];17(24):3965–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18801879

20. Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM, et al. Gene expression elucidates functional impact of polygenic risk for schizophrenia. Nat Neurosci [Internet]. 2016;19(11):1442–53. Available from: http://www.nature.com/doifinder/10.1038/nn.4399

21. Lee CS, Buttitta L, Fan C-M. Evidence that the WNT-inducible growth arrest-specific gene 1 encodes an antagonist of sonic hedgehog signaling in the somite. Proc Natl Acad Sci [Internet]. 2001;98(20):11347–52. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.201418298

22. Segovia J, Zarco N. Gas1 is a pleiotropic regulator of cellular functions: from embryonic development to molecular actions in cancer gene therapy. Mini Rev Med Chem [Internet]. 2014 [cited 2017 Sep 29];14(14):1139–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25429664

23. Liu Y, May NR, Fan C-M. Growth Arrest Specific Gene 1 Is a Positive Growth Regulator for the Cerebellum. Dev Biol. 2001;236(1):30–45.

24. Moberget T, Doan NT, Alnæs D, Kaufmann T, Córdova-Palomera A, Lagerberg T V, et al. Cerebellar volume and cerebellocerebral structural covariance in schizophrenia: a multisite mega-analysis of 983 patients and 1349 healthy controls. Mol Psychiatry [Internet]. 2017 May 16 [cited 2017 Sep 29]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/28507318

25. Li Y, Pawlik B, Elcioglu N, Aglan M, Kayserili H, Yigit G, et al. LRP4 Mutations Alter Wnt/β-Catenin Signaling and Cause Limb and Kidney Malformations in Cenani-Lenz Syndrome. Am J Hum Genet [Internet]. 2010 May 14 [cited 2017 Feb 22];86(5):696–706. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20381006

26. Goltzman D, Hendy GN, White JH. Vitamin D and its receptor during late development. Biochim Biophys Acta - Gene Regul Mech [Internet]. Elsevier B.V.; 2015;1849(2):171–80. Available from: http://dx.doi.org/10.1016/j.bbagrm.2014.05.026

27. Liu BY, Soloviev I, Huang X, Chang P, Ernst JA, Polakis P, et al. Mammary tumor regression elicited by Wnt signaling inhibitor requires IGFBP5. Cancer Res. 2012;72(6):1568–78.

28. Benzler J, Andrews ZB, Pracht C, Stöhr S, Shepherd PR, Grattan DR, et al. Hypothalamic WNT Signalling Is Impaired During Obesity and Reinstated by Leptin Treatment in Male Mice. Endocrinology [Internet]. 2013 Dec [cited 2017 Jan 10];154(12):4737–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24105484

29. Phesse TJ, Buchert M, Stuart E, Flanagan DJ, Faux M, Afshar-Sterle S, et al. Partial inhibition of gp130-Jak-Stat3 signaling prevents Wnt-β-catenin-mediated intestinal tumor growth and regeneration. Sci Signal [Internet]. 2014;7(345):ra92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25270258

30. Panaccione I, Napoletano F, Forte AM, Kotzalidis GD, Del Casale A, Rapinesi C, et al. Neurodevelopment in schizophrenia: the role of the wnt pathways. Curr Neuropharmacol [Internet]. 2013;11(5):535–58. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3763761&tool=pmcentrez&ren

dertype=abstract

31. Mulligan KA, Cheyette BNR. Neurodevelopmental Perspectives on Wnt Signaling in Psychiatry. Mol Neuropsychiatry [Internet]. 2016;2:219–46. Available from: https://www.karger.com/Article/Pdf/453266

32. Singh K. An emerging role for Wnt and GSK3 signaling pathways in schizophrenia. Clin Genet [Internet]. 2013 Jun [cited 2017 Jul 14];83(6):511–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23379509

33. Topol A, Zhu S, Tran N, Simone A, Fang G, Brennand KJ. Altered WNT Signaling in Human Induced Pluripotent Stem Cell Neural Progenitor Cells Derived from Four Schizophrenia Patients. Biol Psychiatry. 2015;29–34.

34. Pierfelice T, Alberi L, Gaiano N. Notch in the Vertebrate Nervous System: An Old Dog with New Tricks. Neuron [Internet]. Elsevier Inc.; 2011;69(5):840–55. Available from: http://dx.doi.org/10.1016/j.neuron.2011.02.031

35. Alberi L, Hoey SE, Brai E, Scotti AL, Marathe S. Notch signaling in the brain: In good and bad times. Ageing Res Rev [Internet]. Elsevier B.V.; 2013;12(3):801–14. Available from: http://dx.doi.org/10.1016/j.arr.2013.03.004

36. Hayward P, Kalmar T, Martinez Arias A. Wnt/Notch signalling and information processing during development. Development [Internet]. 2008;135(3):411–24. Available from: http://dev.biologists.org/cgi/doi/10.1242/dev.000505

37. Rusanescu G, Mao J. Notch3 is necessary for neuronal differentiation and maturation in the adult spinal cord. J Cell Mol Med. 2014;18(10):2103–16.

38. Pajaniappan M, Glober NK, Kennard S, Liu H, Zhao N, Lilly B. Endothelial cells downregulate apolipoprotein D expression in mural cells through paracrine secretion and Notch signaling. AJP Hear Circ Physiol [Internet]. 2011;301(3):H784–93. Available from: http://ajpheart.physiology.org/cgi/doi/10.1152/ajpheart.00116.2011

39. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. Immunity [Internet]. Elsevier Inc.; 2011;35(5):780–91. Available from: http://dx.doi.org/10.1016/j.immuni.2011.08.013

40. Krall AS, Xu S, Graeber TG, Braas D, Christofk HR. Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. Nat Commun [Internet]. Nature Publishing Group; 2016;7:11457. Available from: http://www.nature.com/doifinder/10.1038/ncomms11457

41. Ruzzo EK, Capo-Chichi JM, Ben-Zeev B, Chitayat D, Mao H, Pappas AL, et al. Deficiency of Asparagine Synthetase Causes Congenital Microcephaly and a Progressive Form of Encephalopathy. Neuron. 2013;80(2):429–41.

42. Tabatabaie L, Klomp LW, Berger R, de Koning TJ. l-Serine synthesis in the central nervous system: A review on serine deficiency disorders. Mol Genet Metab [Internet]. Elsevier Inc.; 2010;99(3):256–62. Available from: http://dx.doi.org/10.1016/j.ymgme.2009.10.012

43. Ozeki Y, Pickard BS, Kano SI, Malloy MP, Zeledon M, Sun DQ, et al. A novel balanced chromosomal translocation found in subjects with schizophrenia and schizotypal personality disorder: Altered l-serine level associated with disruption of PSAT1 gene expression. Neurosci Res [Internet]. Elsevier Ireland Ltd and Japan Neuroscience

Society; 2011;69(2):154–60. Available from: http://dx.doi.org/10.1016/j.neures.2010.10.003

44. Huang X, Kong H, Tang M, Lu M, Ding J-H, Hu G. D-Serine regulates proliferation and neuronal differentiation of neural stem cells from postnatal mouse forebrain. CNS Neurosci Ther [Internet]. 2012;18(1):4–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22280157

45. Sultan S, Gebara EG, Moullec K, Toni N. D-serine increases adult hippocampal neurogenesis. Front Neurosci. 2013;7(7 AUG):1–10.

46. Cho SE, Na KS, Cho SJ, Kang SG. Low D-serine levels in schizophrenia: A systematic review and meta-analysis. Neurosci Lett [Internet]. Elsevier Ireland Ltd; 2016;634:42–51. Available from: http://dx.doi.org/10.1016/j.neulet.2016.10.006

47. Selvaraj S, Arnone D, Cappai A, Howes O. Alterations in the serotonin system in schizophrenia: A systematic review and meta-analysis of postmortem and molecular imaging studies. Neurosci Biobehav Rev [Internet]. 2014 Sep [cited 2017 Aug 2];45:233–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24971825

48. Pitychoutis PM, Belmer A, Moutkine I, Adrien J, Maroteaux L. Mice Lacking the Serotonin Htr2B Receptor Gene Present an Antipsychotic-Sensitive Schizophrenic-Like Phenotype. Neuropsychopharmacology [Internet]. 2015 May 4 [cited 2015 Jul 15]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25936642

49. Devroye C, Cathala A, Piazza PV, Spampinato U. The central serotonin 2B receptor as a new pharmacological target for the treatment of dopamine-related neuropsychiatric disorders: Rationale and current status of research. Pharmacol Ther [Internet]. 2017 Jul 27 [cited 2017 Aug 2]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/28757154

Acknowledgements.

This study was supported by a NARSAD Young Investigator Award and a NIMH grant

MH086874 to OVE and NIMH grant MH086873 to JAK.