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Integration of gene expression and GWAS results supports involvement of calcium signaling in Schizophrenia

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ABSTRACT

The number of Genome Wide Association Studies (GWAS) of schizophrenia is rapidly growing. However, the small effect of individual genes limits the number of reliably implicated genes, which are too few and too diverse to perform reliable pathway analysis; hence the biological roles of the genes implicated in schizophrenia are unclear. To overcome these limitations we combine GWAS with genome-wide expression data from human post-mortem brain samples of schizophrenia patients and controls, taking these steps: 1) Identify 36 GWAS-based genes which are expressed in our dataset. 2) Find a cluster of 19 genes with highly correlated expression. We show that this correlation pattern is robust and statistically significant. 3) GO-enrichment analysis of these 19 genes reveals significant enrichment of ion channels and calcium-related processes. This finding (based on analyzing a small number of coherently expressed genes) is validated and enhanced in two ways: First, the emergence of calcium channels and calcium signaling is corroborated by identifying proteins that interact with those encoded by the cluster of 19. Second, extend the 19 cluster genes into 1028 genes, whose expression is highly correlated with the cluster's average profile. When GO-enrichment analysis is performed on this extended set, many schizophrenia related pathways appear, with calcium-related processes enriched with high statistical significance. Our results give further, expression-based validation to GWAS results, support a central role of calcium-signaling in the pathogenesis of schizophrenia, and point to additional pathways potentially related to the disease.

1. Introduction

Studies conducted by the International Schizophrenia Consortium attribute one-third of genetic susceptibility for schizophrenia (estimated to be 80% (Sullivan et al., 2003)) to the collective effect of hundreds of common polygenic variants, each contributing a small effect (Purcell et al., 2009; Gejman et al., 2011).

The results of GWAS (Johnson and O'Donnell, 2009) contain many false positives (Burmeister et al., 2008). Current replicable GWAS results account for only a small percentage of the estimated heritability (Ozomaro et al., 2013) and their systematic biological interpretation is lacking. GWAS results have generated several biological hypotheses, such as involvement of *ZNF804A* through regulation of gene expression (O'Donovan et al., 2008); of infection, through interaction with genes located in the major histocompatibility complex (MHC) region, and involvement of calcium channels, based on two GWAS-derived genes that encode for calcium channel subunits (Ripke et al., 2013). Focusing on a specific GWAS-based variant might be, however, misleading, as each specific variant may be a false positive, and in any case it confers only a small increase of the risk.

Higher-level interpretation of GWAS results in terms of implicated pathways is hindered by the number of reliable GWAS-derived genes (see (Ripke et al., 2013)), too small to yield robust and statistically meaningful pathway enrichment analysis. Ripke et al. (2013) dealt with this limitation by focusing from the outset on the set of SNPs located in genes encoding calcium channel subunits, and found enrichment of SNPS with small *p*-values in this set. Jia et al. (2010) performed a broader search and identified enrichment of pathways related to metabolism of glutamate, apoptosis, inflammation and immune system. In O'Dushlaine et al. (2011), the pathway of cell adhesion passed multiple testing correction. In Schizophrenia Working Group of the Psychiatric Genomics (2014), a multi-stage GWAS of up to 36,989 cases and 113,075 controls, 108 loci met genome-wide significance, 83 of which have not been previously reported. Protein-coding variants played a limited role, consistently with the hypothesis that most associated variants detected by GWAS exert their effects through altering

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gene expression rather than protein structure. Genes encoding voltage-gated calcium channel subunits, *CACNA1C*, *CACNB2* and *CACNA1I*, were found to be significantly associated, extending previous findings implicating members of this family of proteins in schizophrenia.

Expression profiling studies in schizophrenia suggested changes in functional gene groups such as oligodendrocyte and myelin related genes, metabolism, synaptic transmission, GABAergic and glutamatergic pathways (Roussos et al., 2012; Katsel et al., 2005a; Mirnics et al., 2006). Often there is little agreement between different microarray studies regarding which transcripts are differentially expressed in the disease, reflecting the GWAS-based picture of a multifactorial heterogeneous disease, caused by a combination of many genetic and environmental factors. The reported results show only modest changes in genes' expression levels between cases and controls, calling for a more involved and integrated analysis. A model of a complex combination of genetic and environmental factors is concordant with the clinical heterogeneity of the disease (for example, gender difference in the age of onset and outcome) and with delicate structural brain changes that are found in both schizophrenia patients and their relatives (Cooper et al., 2014).

Here we focused on functional changes by analyzing a large-scale genome-wide gene expression dataset of postmortem brain samples, for the identification of biological pathways and processes through which GWAS-derived genes affect schizophrenia. Integration of data from different platforms potentially increases the biological and statistical reliability of the results, reducing significantly the false positives rate resulting from each of the separate analyses. A short list of genes identified by GWAS as relevant to the disease were used as “seed”, and the expression data of these and related genes were analyzed. Correlations between their expression profiles were calculated; high pairwise correlations suggest shared biological pathways. A cluster of genes with high pairwise correlations of expression was identified, and gene ontology (GO) enrichment analysis was performed on the members of the cluster. Since the genes of the cluster have correlated expression, the chance that they belong to the same pathway increases. Indeed we identified significantly enriched pathways—ion channels and, specifically, calcium channel activity.

Since the enrichment analysis was based on a small number of genes, we sought ways to validate this result further. First, we searched for proteins that interact with members of our cluster. The interaction partners were mostly related to calcium signaling; calcium channel subunits and members of the calmodulin pathway emerged. For further validation we extended the list of genes to be searched for enrichment, assembling all genes with high correlation of expression with the average expression profile of the cluster. Enrichment analysis of this extended list identified many pathways that are known to be associated with schizophrenia, and calcium-related pathways re-emerged with high statistical significance.

2. Materials and methods

2.1. Subjects

Human brain samples were obtained from the Brain Bank of the Department of Psychiatry of the Mount Sinai Medical Center (New York, NY)/JJ Peters Veterans Administration Medical Center (Bronx, NY). Dissections were performed blind to diagnosis. All cortical dissections and sample preparation were performed as described previously (Hakak et al., 2001; Katsel et al., 2005a; Katsel et al., 2005b). Brain banking activities were approved by the Institutional Review Board of the Mount Sinai School of Medicine, and written consent for brain donation was obtained from the next-of-kin of all subjects.

Table 1 shows the main characteristics of the population analyzed. All subjects were clinically and neuropathologically assessed as described previously (Purohit et al., 1998).

Table 1
Patients' characteristics. Control samples were derived from subjects with no evidence of dementia, neuro or psychopathology. The cases with schizophrenia were diagnosed by clinical investigators to meet criteria for schizophrenia by DSM-III/IV and to evidence no other comorbid psychopathology.

Characteristics	Schizophrenia	Control
Number of patients	28	22
Sex (M/F)	20/8	8/14
Age (years)	74.2 (11.4)	82.8 (11.7)
Neuritic plaque density (#/mm^2)	1.1 (0.4)	1.3 (0.6)
Cognitive dementia Rating	2.4 (1.7)	0.5 (0.9)
Braak staging	0.3 (0.5)	2.0 (1.2)
Brain pH	6.4 (0.2)	6.6 (0.3)
PM1 (min)	683 (493)	419 (329)

2.2. Gene expression pre-processing

The computational analysis starts with the raw data of Affymetrics HG-U133A arrays, listed in Table 2 by their distribution across 17 brain regions, in cases and controls. Standard MAS-5 algorithm was used for normalization. Then expression levels below 20 were set 20 and log2-transformation was applied. Probe-sets without assigned Affymetrix gene symbols annotation were removed. 12,033 probe-sets were left for the rest of the analysis after filtering (out of 22,283), representing 8542 gene symbols. Probe sets of the same gene were combined. For full details see supplementary Methods.

2.3. Analysis of the expression patterns of the 36 GWAS-based genes

We used the SPIN tool (Tsafrir et al., 2005) to order genes (samples), such that genes (samples) with similar expression pattern are grouped together. SPIN applies an iterative search algorithm, and sorts items into neighborhoods of similar patterns. Then, for each pair of the 36 genes, Pearson correlation of expression, along all 480 samples, was calculated.

2.4. Estimating the statistical significance of the observed correlation pattern

We randomly selected a group of 36 genes and calculated their pairwise correlation values. Repeating this 1000 times we generated

Table 2
Distribution of Affymetrix HG-133A arrays across brain tissues and populations.

Anatomical gr.		Brodmann area	Schizophrenia	Control
Frontal lobe	Anterior prefrontal cortex	BA10	18	14
	Frontal eye fields	BA8	14	12
	Dorsolateral prefrontal cortex	BA46	20	18
	Primary motor cortex	BA4	15	6
	Pars opercularis, part of Broca's area	BA44	15	11
Cingulate gyrus	Anterior cingulate gyrus	BA32	15	17
	Posterior cingulate gyrus	BA23	11	13
Temporal lobe	Hippocampus	HIPP	12	12
	Temporopolar area	BA38	14	14
	Parahippocampal cortex	BA36	14	17
	Inferior temporal gyrus	BA20	17	14
	Middle temporal gyrus	BA21	17	16
	Superior temporal gyrus	BA22	19	14
Parietal lobe	Somatosensory association cortex	BA7	15	13
Occipital lobe	Primary visual cortex (V1)	BA17	13	13
Basal ganglia	Caudate nucleus	CD	15	11
	Putamen	PT	11	10
		Total	255	225

the distribution of the absolute values of the pairwise correlations, and estimated the probability of getting by chance similar or higher absolute correlation values to those of the GWAS-derived genes (see Supplementary Methods).

2.5. Generating an extended correlated gene set for enrichment analysis

First, a cluster of 19 genes, out of the 36 GWAS-derived genes is identified (see Fig. 1). Then the average expression profile of the 19 clustered genes is calculated, and we expand the clustered genes into a list of 1028 genes, whose Pearson correlation with the average profile exceeds 0.5. Expansion of the 19 genes into a longer list is necessary in order to apply pathway analysis in a robust and statistically meaningful way.

2.6. Pathway analysis using DAVID.

The DAVID tool (Dennis et al., 2003) was applied to compute enrichment of Gene Ontology (GO) annotations. Terms that passed 5% False Discovery Rate (FDR) (Benjamini and Hochberg, 1995) were selected. Similar terms were grouped into clusters using DAVID functional annotation clustering (Huang da et al., 2009).

2.7. Protein interaction analysis using STRING

STRING database (Szklarczyk et al., 2011) was applied to detect protein interactions for a given gene. The parameters used: confidence score: 0.9 (highest, focusing on the most confident interactions), using all prediction methods (default).

3. Results

3.1. 36 GWAS-derived genes.

The recent GWAS analysis of (Schizophrenia Working Group of the Psychiatric Genomics, 2014) identified 108 loci that have potential roles in schizophrenia, with genome-wide significance. 58 of these are associated with a single gene symbol. In order to control for background noise, we applied various quality filters (see Supplementary Methods); 36 genes, listed in Table 3, passed these filters and were expressed at a high enough level in our dataset.

Table 3
36 schizophrenia GWAS-derived genes (from (Schizophrenia Working Group of the Psychiatric Genomics, 2014); out of 58 loci that are associated with a single gene symbol), that appear in our dataset and are expressed above threshold.

	Gene symbol
1	ZNF804A
2	PJA1
3	GPM6A
4	FAM5B
5	GRAMD1B
6	CYP26B1
7	KCNV1
8	SATB2
9	CACNB2
10	SNAP91
11	NLGN4X
12	CNKSR2
13	MEF2C
14	GRIA1
15	FUT9
16	CUL3
17	CACNA1C
18	GRIN2A
19	ATP2A2
20	MAD1L1
21	TBC1D5
22	TCF4
23	MMP16
24	CACNA1I
25	TLE3
26	BCL11B
27	TLE1
28	DRD2
29	PRKD1
30	PODXL
31	SNX19
32	ZNF536
33	MAN2A1
34	SLC39A8
35	GRM3
36	GALNT10

3.2. Schizophrenia associated genes exhibit robust and statistically significant high correlations of their expression profiles

Expression values (see Methods) of the 36 GWAS-based genes were measured in 480 samples from 28 schizophrenia patients and 22

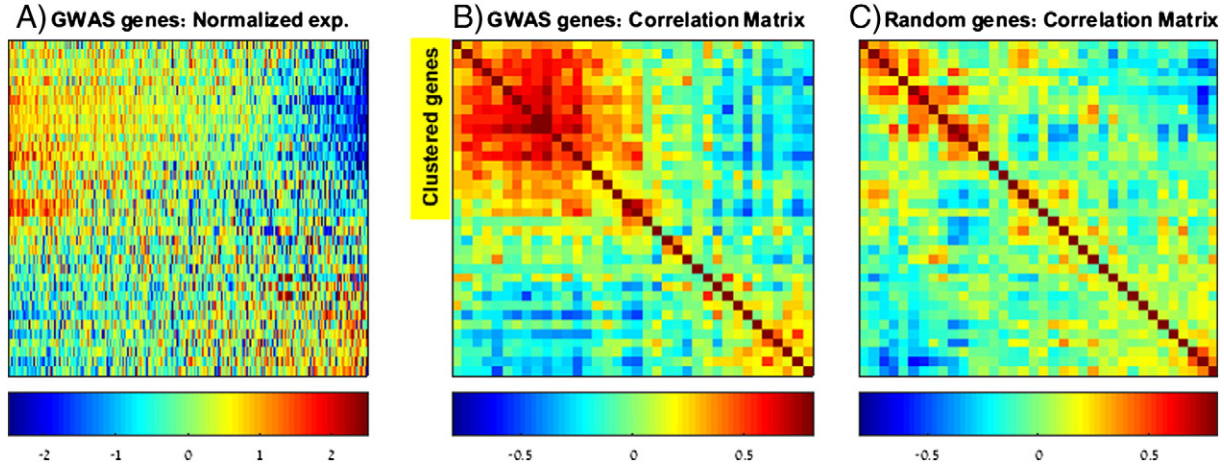

Fig. 1. 36 schizophrenia associated genes: Pearson correlation pattern. A): Centered and normalized expression of the 36 GWAS-derived genes. Each row represents a gene; each column represents a sample. The color in each entry (y,x), where $x = 1, \ldots, 480$ (number of samples), represents the expression of gene y in sample x. Genes and samples are ordered by SPIN (Tsafrir et al., 2005); gene ordering can be read off Table 3. B): Pearson correlation matrix of the expression of the 36 GWAS-derived genes. The color in each entry (y,x), where x,$y = 1, \ldots, 36$ (number of genes) represents the Pearson correlation between the expression of genes y and x, along all 480 samples. The genes are in the same order as in A. The background yellow color along the y-axis indicates genes that are clustered together. C): Pearson correlation matrix of a random group of 36 genes (see legend above for the colors).

Table 4
Gene Ontology enriched terms among the 19 genes of our largest cluster. Terms are grouped into clusters (in this case one cluster) according to DAVID Functional annotation clustering. Inside each cluster, terms are ordered by ascending order of their enrichment *P*-values.

Cluster number and score	Category	Term	Num genes	*P* value	FDR
Cluster 1, enrichment score: 1.96					
	MF	GO:0046873 ~ metal ion transmembrane transporter activity	6	<0.0001	0.25%
	MF	GO:0005216 ~ ion channel activity	6	<0.0001	0.27%
	MF	GO:0022838 ~ substrate specific channel activity	6	<0.0001	0.21%
	MF	GO:0015267 ~ channel activity	6	<0.0001	0.19%
	MF	GO:0022803 ~ passive transmembrane transporter activity	6	<0.0001	0.15%
	MF	GO:0005262 ~ calcium channel activity	4	<0.0001	0.16%
	MF	GO:0005261 ~ cation channel activity	5	0.00022	0.33%
	MF	GO:0022836 ~ gated channel activity	5	0.00035	0.45%
	MF	GO:0022843 ~ voltage-gated cation channel activity	4	0.00059	0.67%
	MF	GO:0005244 ~ voltage-gated ion channel activity	4	0.0013	1.40%
	MF	GO:0022832 ~ voltage-gated channel activity	4	0.0013	1.40%

CC: cellular component; BP: biological process; MF: molecular function.

controls, collected from 17 Brodmann areas (see Methods) and are presented as a heatmap in Fig. 1A. The rows (genes) and columns (samples) were ordered by SPIN (Tsafrir et al., 2005). Pearson correlation coefficients between each pair of the 36 genes' expression profiles over all samples are presented in Fig. 1B (the genes are ordered as in Fig. 1A and can be read off Table 3). Clearly, correlation absolute values are relatively high; 19 genes (highlighted by a yellow sidebar) are grouped into one cluster with particularly high pairwise correlation values. To test robustness of this gene cluster, we repeated the analysis for several subgroups of the samples. In particular, we checked whether our results are biased by the uneven distribution of gender among the patients and controls. As can be seen in Supplementary Figs. 2S and 3S, the described correlation pattern is present for both the schizophrenia and control samples, in both females and males, and in each of the 17 Brodmann areas included in our analysis. Thus, the signal is robust, and is not restricted to a specific subgroup of the samples.

We estimated the statistical significance of the correlation pattern of the GWAS-derived genes generating random groups of 36 genes (see Materials and methods 2.3, Supplementary Methods and Fig. 1S). A typical correlation matrix for such a group is shown in Fig. 1C. The *p*-value of correlations like those of the GWAS-derived genes was less than 0.01, indicating that there is a biological basis for the observed correlations; plausibly, genes with such correlations participate in common biological pathways. From this point we focus on the 19 genes of the cluster.

Only 3 out of the 19 genes (*FUT*, *GRAMD1B* and *CNKSR1*) show statistically significant differential expression in schizophrenia versus controls (two-sided *t*-test, see Supplementary Table 1S), and even these had fold change ≤ 1.1. The clinical significance of such modest changes in expression is unclear, but they are concordant with the accepted notion that in schizophrenia each contributor has a small effect. Therefore, rather than focusing on specific genes, we analyze

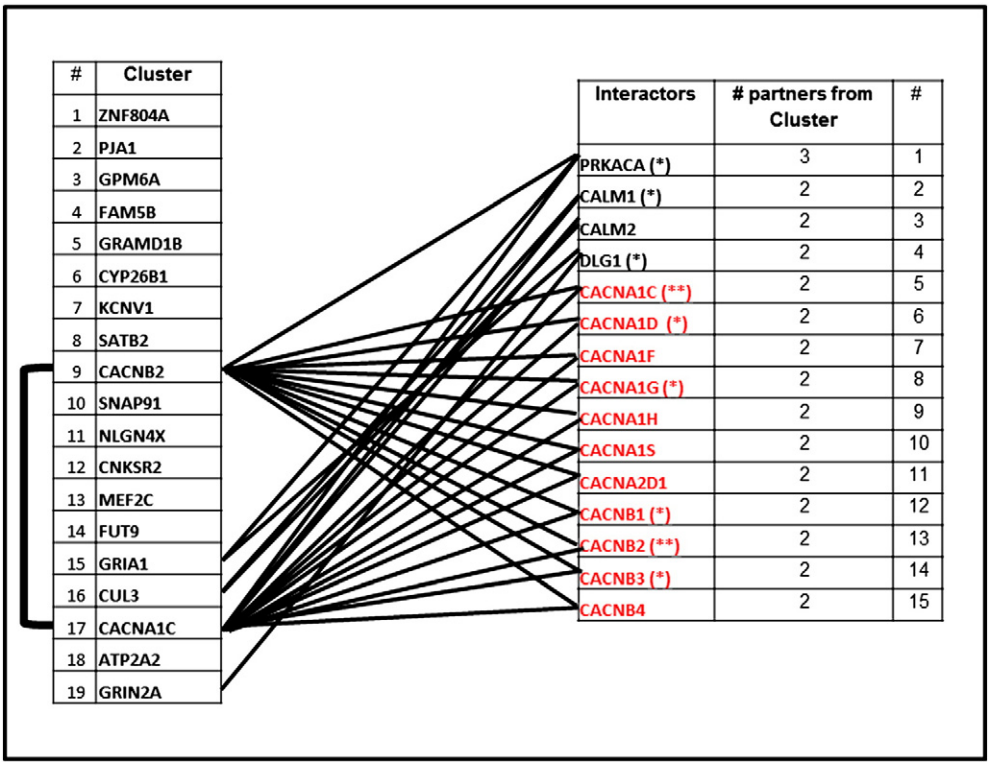

Fig. 2. STRING database results of genes that interact with the 19 cluster genes. Each line that connects two genes represents an interaction identified by the STRING database. Of the 19 cluster genes (listed on the left), only two had direct interaction. Identified interactors are listed to the right: those present in our expression dataset are marked by (*). *CACNA1C* and *CACNB2* are self-interacting; hence appear in both lists and marked by (**). Genes encoding for calcium channel subunits are colored red.

the genes as a group, to reveal pathways involved in the pathogenesis of schizophrenia.

3.3. Search for biological pathways in which schizophrenia associated genes play a significant role

We applied DAVID pathway analysis (Materials and methods 2.6) to our group of interest of the 19 cluster genes. Since usually the number of genes in the tested group has to be relatively large in order to yield good statistics, we were surprised to get statistically significant enrichments for our group of 19 genes. As can be seen in Table 4, GO terms that are connected to ion channel activity (specifically to calcium), were enriched at a statistically significant level (FDR < 5%). This finding is consistent with GWAS analysis (Ripke et al., 2013). We noted that our results (based on 19 genes) were not robust against omitting the two genes, *CACNB2* and *CACNA1C* (encoding calcium channel subunits); hence independent validation of our results is needed.

To further explore the hypothesis that calcium-signaling pathways play a role in schizophrenia, we used the STRING database (Materials and methods 2.7), searching for proteins that interact with one of the 19 of the cluster. As seen in Fig. 2, direct intra-cluster interaction was detected only between the two calcium channel subunits *CACNB2* and *CACNA1C*. We did find several relevant indirect interactions, between pairs from the cluster, mediated by a shared protein, that interacts with both cluster members. Most indirect interactions involve proteins of calcium channel subunits (colored red in Fig. 2); these interact mainly with *CACNB2* and *CACNA1C* from the cluster, which makes sense, as these subunits form the calcium channel. Notably, however, 3 out of the 4 interactors that don't encode for calcium channel subunits, *PRKACA* and *CALM1*, *CALM2* (encoding Calmodulin), are involved in calcium signaling. Calmodulin is required for many calcium-sensitive signal transduction pathways (Hidaka and Ishikawa, 1992) and PRKACA (Protein Kinase, cAMP-Dependent, Catalytic, Alpha) is involved in calcium signaling, specifically in the Calmodulin-pathway (see (Dash et al., 1991), and GeneCards (www.genecards.org (Belinky et al., 2013)). Thus, these findings support involvement of the 19 GWAS-derived genes in calcium-related pathways. Of the 15 interactor genes 7 (marked by (*) in Fig. 2) are present in our expression dataset. We added these 7 to the 36 GWAS-derived genes, and show in Fig. 3 the pairwise correlations of expression of all 43. It can be seen that *PRKACA*, *CALM1* and the genes encoding calcium channel subunits cluster together with the 19 GWAS genes, further supporting our finding, that the 19 GWAS-derived genes cooperate in calcium-related biological pathways.

To strengthen the statistical reliability of our results, we enlarged the set of genes tested for enrichment, expanding the 19 clustered genes into a list of 1028 genes, whose Pearson correlation with the "cluster profile" exceeds 0.5, see Fig. 4. From such a longer list of genes standard pathway analysis tools can provide robust and statistically meaningful results.

Pathway analysis using DAVID (Materials and methods 2.6) found many enriched GO terms in our extended list of 1028 genes, see Table 2S. Similar terms are grouped into 48 clusters using DAVID functional annotation clustering (Huang da et al., 2009). Representative terms (listed according to the cluster number to which they belong) from each cluster appear in Table 5.

Supporting the observations made above, the GO terms "calcium ion transport" and "calcineurin complex" (calcineurin is a calcium/calmodulin protein phosphatase), are highly enriched in the extended list based on the 19 clustered genes (listed 22, 43 in Table 5). More general nervous system terms that were implicated in schizophrenia, such as regulation of neuron projection development, axon and regulation of synaptic transmission were also found to be enriched. There are also enriched terms that are more specific to the central nervous system and are thought to be connected to schizophrenia, such as behavior, learning or memory and glutamate receptor activity (see Table 5). The large number of enriched pathways, including known schizophrenia-related pathways, indicates that the 1028 highly-correlated genes cooperate in biological pathways that might be activated in concordance in the pathogenesis of schizophrenia.

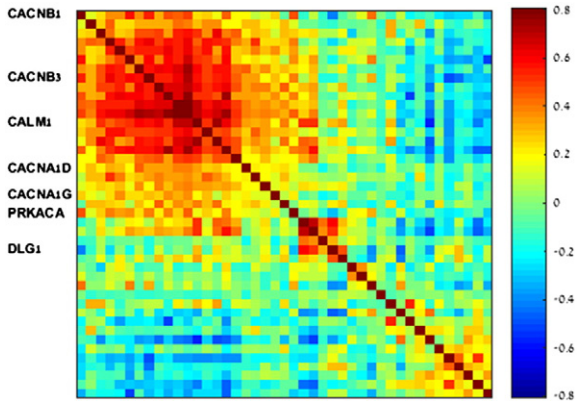

Fig. 3. Pearson correlation matrix of 36 GWAS-derived genes and 7 interactor genes, resulting from STRING database analysis: 7 interactor genes (see text and Fig. 2) that are present in our expression dataset were added to the 36 GWAS-derived genes. The Pearson correlation matrix between these 43 genes (see caption of Fig. 2B) is shown. The genes were ordered by SPIN (Tsafrir et al., 2005). The gene symbol of each gene of the 7 interactors appears next to the row that represents it.

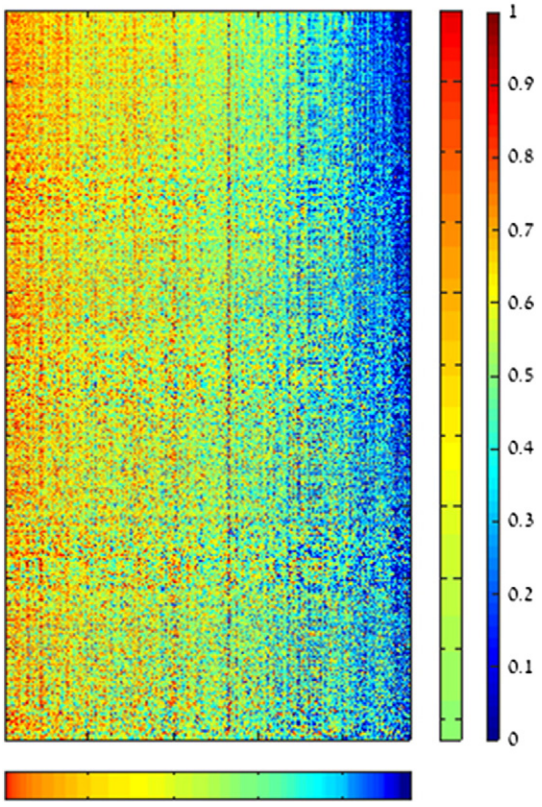

Fig. 4. Expression matrix of the extended list of highly-correlated genes. Normalized expression of the extended group of 1,028 genes with Pearson correlation value > 0.5, measured between each gene's expression pattern and the "core" group average pattern. Each row represents a gene (1,028 genes); each column represents a sample (480 samples). The normalized expression values are represented by the same color scheme as shown on the colorbar below Fig 1A. The average value of the expression taken for each sample over the 19 cluster genes is represented in the bar below the expression matrix (the columns were ordered according to these average expression values, low values on the left side). The vertical color bar to the right of the matrix presents the value of the Pearson correlation of each gene with the average pattern (the rightmost vertical color bar defines the value represented by each color). The rows were ordered according to the value of the correlation coefficient, with low values (starting at 0.5) at bottom.

4. Discussion

Altered functionality of a group of genes centered on a specific pathway might have a synergistic effect on the risk for the development of a disease. Thus, pathway analysis is important for the biological interpretation of GWAS results. On the one hand, however, the number of genes reliably identified by GWAS as disease associated is small; on the other, lowering the threshold on GWAS-based identification introduces many false positives. Both situations hinder derivation of robust and statistically meaningful results from pathway analysis.

By combining schizophrenia GWAS results with gene expression analysis we were able to obtain robust and statistically significant pathway analysis. Our main finding implicates calcium ion transport and calcium signaling in the pathogenesis of the disease.

Involvement of calcium signaling in schizophrenia was previously claimed, using different kinds of evidence. It was proposed that altered calcium signaling may constitute the central unifying molecular pathology in schizophrenia (Lidow, 2003). In addition, evidence for the ability of antipsychotic drugs to affect calcium signaling was presented. Giegling et al. (2010) presented converging genetic evidence for the contribution of genes potentially related to alterations in intracellular calcium-homeostasis to the risk of schizophrenia. Moreover, in Cross-Disorder Group of the Psychiatric Genomics (2013), two L-type voltage-gated calcium channel subunits, *CACNA1C* and *CACNB2* show cross-disorder association, not specific to schizophrenia. Finally, both GWAS data (Ripke et al., 2013) and analysis of rare disruptive mutations in schizophrenia (Purcell et al., 2014) suggest the involvement of voltage-gated calcium channels.

Table 5
19 Clustered Gene Ontology terms enriched in the extended list of 1028 genes (see text). Terms are ordered by ascending order of their enrichment *P*-values.

Cluster number and score	Category	Term	Num genes	PValue	Benjamini
1	CC	GO:0045202 ~ synapse	76	<0.0001	<0.0001%
2	CC	GO:0030424 ~ axon	39	<0.0001	<0.0001%
3	CC	GO:0044456 ~ synapse part	57	<0.0001	<0.0001%
4	CC	GO:0043005 ~ neuron projection	73	<0.0001	<0.0001%
5	BP	GO:0019226 ~ transmission of nerve impulse	63	<0.0001	<0.0001%
6	MF	GO:0000166 ~ nucleotide binding	196	<0.0001	<0.0001%
7	CC	GO:0031982 ~ vesicle	86	<0.0001	<0.0001%
8	BP	GO:0006091 ~ generation of precursor metabolites and energy	53	<0.0001	<0.0001%
9	BP	GO:0031175 ~ neuron projection development	43	<0.0001	<0.0001%
10	BP	GO:0006836 ~ neurotransmitter transport	20	<0.0001	<0.01%
11	BP	GO:0050804 ~ regulation of synaptic transmission	27	<0.0001	<0.001%
12	BP	GO:0007017 ~ microtubule-based process	38	<0.0001	<0.001%
13	BP	GO:0010970 ~ microtubule-based transport	12	<0.0001	<0.01%
14	BP	GO:0016192 ~ vesicle-mediated transport	68	<0.0001	<0.001%
15	BP	GO:0006796 ~ phosphate metabolic process	96	<0.0001	<0.01%
16	BP	GO:0046907 ~ intracellular transport	73	<0.0001	<0.001%
17	BP	GO:0034613 ~ cellular protein localization	45	<0.0001	0.20%
18	CC	GO:0000267 ~ cell fraction	99	<0.0001	0.02%
19	MF	GO:0003924 ~ GTPase activity	31	<0.0001	0.02%
20	BP	GO:0006811 ~ ion transport	89	<0.0001	<0.0001%
21	BP	GO:0007610 ~ behavior	51	<0.0001	0.08%
22	BP	GO:0006816 ~ calcium ion transport	22	<0.0001	0.23%
23	BP	GO:0007611 ~ learning or memory	22	<0.0001	<0.01%
24	CC	GO:0031090 ~ organelle membrane	106	<0.0001	<0.001%
25	CC	GO:0044459 ~ plasma membrane part	184	<0.0001	<0.001%
26	BP	GO:0006096 ~ glycolysis	13	<0.0001	0.05%
27	BP	GO:0060079 ~ regulation of excitatory postsynaptic membrane potential	7	0.00051	1.50%
28	MF	GO:0016247 ~ channel regulator activity	14	<0.0001	0.08%
29	BP	GO:0017157 ~ regulation of exocytosis	10	<0.0001	0.33%
30	BP	GO:0008038 ~ neuron recognition	9	<0.0001	0.06%
31	CC	GO:0044429 ~ mitochondrial part	57	0.0004	0.36%
32	BP	GO:0048488 ~ synaptic vesicle endocytosis	7	<0.0001	0.26%
33	CC	GO:0033176 ~ proton-transporting V-type ATPase complex	10	<0.0001	<0.01%
34	CC	GO:0033267 ~ axon part	12	0.00027	0.27%
35	BP	GO:0060052 ~ neurofilament cytoskeleton organization	6	<0.0001	0.12%
36	BP	GO:0016079 ~ synaptic vesicle exocytosis	7	0.00012	0.39%
37	BP	GO:0050808 ~ synapse organization	12	0.00064	1.80%
38	BP	GO:0003001 ~ generation of a signal involved in cell-cell signaling	18	<0.0001	0.03%
39	BP	GO:0010975 ~ regulation of neuron projection development	14	0.00016	0.50%
40	CC	GO:0005905 ~ coated pit	11	0.0002	0.21%
41	BP	GO:0016265 ~ death	62	0.0017	4%
42	BP	GO:0051258 ~ protein polymerization	10	0.0017	4%
43	CC	GO:0005955 ~ calcineurin complex	4	0.0019	1.40%
44	MF	GO:0046933 ~ hydrogen ion transporting ATP synthase activity, rotational mechanism	7	0.00017	0.47%
45	BP	GO:0007010 ~ cytoskeleton organization	47	<0.0001	0.20%
46	MF	GO:0005086 ~ ARF guanyl-nucleotide exchange factor activity	6	0.0021	4%
47	BP	GO:0009132 ~ nucleoside diphosphate metabolic process	6	0.0022	5%
48	BP	GO:0043242 ~ negative regulation of protein complex disassembly	9	0.0021	4.70%
49	MF	GO:0008066 ~ glutamate receptor activity	8	0.0015	3.20%
50	BP	GO:0043254 ~ regulation of protein complex assembly	14	0.0019	4.40%
51	BP	GO:0006090 ~ pyruvate metabolic process	10	0.00054	1.50%
52	BP	GO:0009132 ~ nucleoside diphosphate metabolic process	6	0.0022	5%
53	BP	GO:0009060 ~ aerobic respiration	10	0.00012	0.40%
54	BP	GO:0045333 ~ cellular respiration	17	0.00013	0.41%

CC: cellular component; BP: biological process; MF: molecular function.

Our analysis of a large gene list, derived by extending the clustered GWAS-derived genes on the basis of expression data implicated additional pathways, many of which are believed to be involved in schizophrenia. These include calcineurin complex, regulation of synaptic transmission, and learning or memory. Interestingly, some of these pathways are closely linked to calcium signaling. Calcineurin is a calcium/calmodulin protein phosphatase. Calcineurin-knockout mice were shown to have impaired working memory, decreased social interactions and increased locomotor activity, a phenotype consisted with schizophrenia (Miyakawa et al., 2003). Calcium influx was shown to control synaptic transmission (Borst and Sakmann, 1996) and long-term potentiation (LTP), which is one of the main cellular models of learning and memory, and is triggered by a transient increase in intracellular calcium concentration (Nicoll and Malenka, 1999)

Schizophrenia is a multifactorial disease, whose pathogenesis probably involves several biological pathways, comprising a complex network. Our analysis points at calcium signaling pathways and many additional biological pathways, of which several have already been linked to schizophrenia. Possibly, these pathways act in concordance, as suggested by the high correlation of their expression patterns. An attractive hypothesis, supported by our results, is that calcium signaling is a key factor in this network. However, further study is needed to deepen our understanding of the mechanisms by which calcium signaling and the additional pathways we found are involved in the pathogenesis of schizophrenia. Such studies might answer the question, which of the pathways serves as a key factor in the pathogenesis of the disorder.

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Contributors

L. Hertzberg designed and performed the analysis and wrote the manuscript. E. Domany supervised the computational analysis and interpretation of the results and the writing of the manuscript. V. Haroutunian provided the gene expression and clinical data, and supervised the biological interpretation of the results and the writing of manuscript. P. Kastel contributed to the generation of the expression data and commented on the manuscript. P. Roussos contributed to the generation of the expression data and commented on the manuscript. All authors contributed to and have approved the final version.

Conflict of interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2015.02.001.

References

Belinky, F., Bahir, I., Stelzer, G., Zimmerman, S., Rosen, N., Nativ, N., Dalah, I., Iny Stein, T., Rappaport, N., Mituyama, T., Safran, M., Lancet, D., 2013. Non-redundant compendium of human ncRNA genes in GeneCards. Bioinformatics 29 (2), 255–261.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. 57 (1), 289–300.

Borst, J.G., Sakmann, B., 1996. Calcium influx and transmitter release in a fast CNS synapse. Nature 383 (6599), 431–434.

Burmeister, M., McInnis, M.G., Zollner, S., 2008. Psychiatric genetics: progress amid controversy. Nat. Rev. Genet. 9 (7), 527–540.

Cooper, D., Barker, V., Radua, J., Fusar-Poli, P., Lawrie, S.M., 2014. Multimodal voxel-based meta-analysis of structural and functional magnetic resonance imaging studies in those at elevated genetic risk of developing schizophrenia. Psychiatry Res. 221 (1), 69–77.

Cross-Disorder Group of the Psychiatric Genomics, C, 2013. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 381 (9875), 1371–1379.

Dash, P.K., Karl, K.A., Colicos, M.A., Prywes, R., Kandel, E.R., 1991. cAMP response element-binding protein is activated by Ca2+/calmodulin- as well as cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. U. S. A. 88 (11), 5061–5065.

Dennis Jr., G., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., Lempicki, R.A., 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 4 (5), P3.

Gejman, P.V., Sanders, A.R., Kendler, K.S., 2011. Genetics of schizophrenia: new findings and challenges. Annu. Rev. Genomics Hum. Genet. 12, 121–144.

Giegling, I., Genius, J., Benninghoff, J., Rujescu, D., 2010. Genetic findings in schizophrenia patients related to alterations in the intracellular Ca-homeostasis. Prog. Neuropsychopharmacol. Biol. Psychiatry 34 (8), 1375–1380.

Hakak, Y., Walker, J.R., Li, C., Wong, W.H., Davis, K.L., Buxbaum, J.D., Haroutunian, V., Fienberg, A.A., 2001. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 98 (8), 4746–4751.

Hidaka, H., Ishikawa, T., 1992. Molecular pharmacology of calmodulin pathways in the cell functions. Cell Calcium 13 (6–7), 465–472.

Huang da, W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4 (1), 44–57.

Jia, P., Wang, L., Meltzer, H.Y., Zhao, Z., 2010. Common variants conferring risk of schizophrenia: a pathway analysis of GWAS data. Schizophr. Res. 122 (1–3), 38–42.

Johnson, A.D., O'Donnell, C.J., 2009. An open access database of genome-wide association results. BMC Med. Genet. 10, 6.

Katsel, P., Davis, K.L., Gorman, J.M., Haroutunian, V., 2005a. Variations in differential gene expression patterns across multiple brain regions in schizophrenia. Schizophr. Res. 77 (2–3), 241–252.

Katsel, P., Davis, K.L., Haroutunian, V., 2005b. Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study. Schizophr. Res. 79 (2–3), 157–173.

Lidow, M.S., 2003. Calcium signaling dysfunction in schizophrenia: a unifying approach. Brain Res. Brain Res. Rev. 43 (1), 70–84.

Mirnics, K., Levitt, P., Lewis, D.A., 2006. Critical appraisal of DNA microarrays in psychiatric genomics. Biol. Psychiatry 60 (2), 163–176.

Miyakawa, T., Leiter, L.M., Gerber, D.J., Gainetdinov, R.R., Sotnikova, T.D., Zeng, H., Caron, M.G., Tonegawa, S., 2003. Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 100 (15), 8987–8992.

Nicoll, R.A., Malenka, R.C., 1999. Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. Ann. N. Y. Acad. Sci. 868, 515–525.

O'Donovan, M.C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., Hamshere, M., Carroll, L., Georgieva, L., Dwyer, S., Holmans, P., Marchini, J.L., Spencer, C.C., Howie, B., Leung, H.T., Hartmann, A.M., Moller, H.J., Morris, D.W., Shi, Y., Feng, G., Hoffmann, P., Propping, P., Vasilescu, C., Maier, W., Rietschel, M., Zammit, S., Schumacher, J., Quinn, E.M., Schulze, T.G., Williams, N.M., Giegling, I., Iwata, N., Ikeda, M., Darvasi, A., Shifman, S., He, L., Duan, J., Sanders, A.R., Levinson, D.F., Gejman, P.V., Cichon, S., Nothen, M.M., Gill, M., Corvin, A., Rujescu, D., Kirov, G., Owen, M.J., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Cloninger, C.R., 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat. Genet. 40 (9), 1053–1055.

O'Dushlaine, C., Kenny, E., Heron, E., Donohoe, G., Gill, M., Morris, D., International Schizophrenia, C, Corvin, A., 2011. Molecular pathways involved in neuronal cell adhesion and membrane scaffolding contribute to schizophrenia and bipolar disorder susceptibility. Mol. Psychiatry 16 (3), 286–292.

Ozomaro, U., Wahlestedt, C., Nemeroff, C.B., 2013. Personalized medicine in psychiatry: problems and promises. BMC Med. 11, 132.

Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F., Sklar, P., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460 (7256), 748–752.

Purcell, S.M., Moran, J.L., Fromer, M., Ruderfer, D., Solovieff, N., Roussos, P., O'Dushlaine, C., Chambert, K., Bergen, S.E., Kahler, A., Duncan, L., Stahl, E., Genovese, G., Fernandez, E., Collins, M.O., Komiyama, N.H., Choudhary, J.S., Magnusson, P.K., Banks, E., Shakir, K., Garimella, K., Fennell, T., DePristo, M., Grant, S.G., Haggarty, S.J., Gabriel, S., Scolnick, E.M., Lander, E.S., Hultman, C.M., Sullivan, P.F., McCarroll, S.A., Sklar, P., 2014. A polygenic burden of rare disruptive mutations in schizophrenia. Nature 506 (7487), 185–190.

Purohit, D.P., Perl, D.P., Haroutunian, V., Powchik, P., Davidson, M., Davis, K.L., 1998. Alzheimer disease and related neurodegenerative diseases in elderly patients with schizophrenia: a postmortem neuropathologic study of 100 cases. Arch. Gen. Psychiatry 55 (3), 205–211.

Ripke, S., O'Dushlaine, C., Chambert, K., Moran, J.L., Kahler, A.K., Akterin, S., Bergen, S.E., Collins, A.L., Crowley, J.J., Fromer, M., Kim, Y., Lee, S.H., Magnusson, P.K., Sanchez, N., Stahl, E.A., Williams, S., Wray, N.R., Xia, K., Bettella, F., Borglum, A.D., Bulik-Sullivan, B.K., Cormican, P., Craddock, N., de Leeuw, C., Durmishi, N., Gill, M., Golimbet, V., Hamshere, M.L., Holmans, P., Hougaard, D.M., Kendler, K.S., Lin, K., Morris, D.W., Mors, O., Mortensen, P.B., Neale, B.M., O'Neill, F.A., Owen, M.J., Milovancevic, M.P., Posthuma, D., Powell, J., Richards, A.L., Riley, B.P., Ruderfer, D., Rujescu, D., Sigurdsson, E., Silagadze, T., Smit, A.B., Stefansson, H., Steinberg, S., Suvisaari, J., Tosato, S., Verhage, M., Walters, J.T., Levinson, D.F., Gejman, P.V., Laurent, C., Mowry, B.J., O'Donovan, M.C., Pulver, A.E., Schwab, S.G., Wildenauer, D.B., Dudbridge, F., Shi, J., Albus, M., Alexander, M., Campion, D., Cohen, D., Dikeos, D., Duan, J., Eichhammer, P., Godard, S., Hansen, M., Lerer, F.B., Liang, K.Y., Maier, W., Mallet, J., Nertney, D.A., Nestadt, G., Norton, N., Papadimitriou, G.N., Ribble, R.,

Sanders, A.R., Silverman, J.M., Walsh, D., Williams, N.M., Wormley, B., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Arranz, M.J., Bakker, S., Bender, S., Bramon, E., Collier, D., Crespo-Facorro, B., Hall, J., Iyegbe, C., Jablensky, A., Kahn, R.S., Kalaydjieva, L., Lawrie, S., Lewis, C.M., Linszen, D.H., Mata, I., McIntosh, A., Murray, R.M., Ophoff, R.A., Van Os, J., Walshe, M., Weisbrod, M., Wiersma, D., Donnelly, P., Barroso, I., Blackwell, J.M., Brown, M.A., Casas, J.P., Corvin, A.P., Deloukas, P., Duncanson, A., Jankowski, J., Markus, H.S., Mathew, C.G., Palmer, C.N., Plomin, R., Rautanen, A., Sawcer, S.J., Trembath, R.C., Viswanathan, A.C., Wood, N.W., Spencer, C.C., Band, G., Bellenguez, C., Freeman, C., Hellenthal, G., Giannoulatou, E., Pirinen, M., Pearson, R.D., Strange, A., Su, Z., Vukcevic, D., Langford, C., Hunt, S.E., Edkins, S., Gwilliam, R., Blackburn, H., Bumpstead, S.J., Dronov, S., Gillman, M., Gray, E., Hammond, N., Jayakumar, A., McCann, O.T., Liddle, J., Potter, S.C., Ravindrarajah, R., Ricketts, M., Tashakkori-Ghanbaria, A., Waller, M.J., Weston, P., Widaa, S., Whittaker, P., McCarthy, M.I., Stefansson, K., Scolnick, E., Purcell, S., McCarroll, S.A., Sklar, P., Hultman, C.M., Sullivan, P.F., Weston, P., Widaa, S., Whittaker, P., McCarthy, M.I., Stefansson, K., Scolnick, E., Purcell, S., McCarroll, S.A., Sklar, P., Hultman, C.M., Sullivan, P.F., Weston, P., Widaa, S., Whittaker, P., McCarthy, M.I., Stefansson, K., Scolnick, E., Purcell, S., McCarroll, S.A., Sklar, P., Hultman, C.M., Sullivan, P.F., 2013. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat. Genet. 45 (10), 1150–1159.

Roussos, P., Katsel, P., Davis, K.L., Siever, L.J., Haroutunian, V., 2012. A system-level transcriptomic analysis of schizophrenia using postmortem brain tissue samples. Arch. Gen. Psychiatry 69 (12), 1205–1213.

Schizophrenia Working Group of the Psychiatric Genomics, C, 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511 (7510), 421–427.

Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch. Gen. Psychiatry 60 (12), 1187–1192.

Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., Doerks, T., Stark, M., Muller, J., Bork, P., Jensen, L.J., von Mering, C., 2011. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res. 39 (Database issue), D561–D568.

Tsafrir, D., Tsafrir, I., Ein-Dor, L., Zuk, O., Notterman, D.A., Domany, E., 2005. Sorting points into neighborhoods (SPIN): data analysis and visualization by ordering distance matrices. Bioinformatics 21 (10), 2301–2308.