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The effects of siRNA-mediated RGS4 gene silencing on the whole genome transcription profile: implications for schizophrenia

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Monika Vrajová [1,2], Soňa Peková [3], Jiří Horáček [1,2], Cyril Höschl [1,2]

1. Prague Psychiatric Center, Czech Republic
2. 3rd Medical Faculty of Charles University, Prague, Czech Republic
3. Laboratory of Molecular Diagnostics, Chambon Inc., Prague, Czech Republic.

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Abstract

The regulator of G-protein signaling (RGS) molecules represent a class of proteins that modulate the signaling activity of G-protein coupled receptors. Regulator of G-protein signaling 4 (RGS4) is of particular interest in schizophrenia due to reported downregulation of RGS4 transcripts in schizophrenia as well as a connection between RGS4 and a number of receptors implicated in schizophrenia. The mechanism of RGS4 involvement in the pathophysiology of this illness is not clear.

To elucidate thise role of RGS4 in pathophysiology of schizophrenia, we silenced RGS4 using siRNAs in human neuroblastoma cell lines and we studied the effects of differential RGS4 expression by microarray.

The cell lines with downregulated expression of RGS4 showed 67 genes with changed expression (30 underexpressed and 37 overexpressed). We have detected three subgroups of genes which might be implicated in schizophrenia pathophysiology: histone genes, which suggest epigenetic me-

Correspondence to: Monika Vrajová, MSc., Prague Psychiatric Center, Ustavni 91, CZ-181 03 Prague 8, Czech Republic. TEL: +420-26600173; FAX: +420-266003160; E-MAIL: vrajova@pcp.lf3.cuni.cz

chanisms of the disease; genes for transcription factors associated with other genes relevant to schizophrenia pathology (BDNF and DISCI1) and a heterogeneous group containing genes for G-proteins (GPR50 and GPR64) and calcium binding proteins.

INTRODUCTION

The genetic mechanisms of schizophrenia are studied largely with the aim to detect genetic variations associated with this illness. There is evidence to suggest an association between genetic polymorphisms of the regulator of G-protein signaling 4 (RGS4) and schizophrenia (Chowandri 2002). The gene for RGS4 has been mapped to the schizophrenia susceptibility locus 1q23 and several single polymorphisms have been associated with the disease (Chowandri *et al.* 2008). RGS4 is highly expressed in the human prefrontal cortex (PFC), an important region of dysfunction in schizophrenia (Erdeley *et al.* 2004). However, analogously to other studies of candidate genes for schizophrenia the association between RGS4 and schizophrenia have suffered from a lack of consistency (Chowandri 2002).

Gene expression profiling in post-mortem tissue from patients with schizophrenia has revealed significant downregulation of the RGS4 transcript. This decrease appears to be selective for this type of RGS and for the brain tissue of patients with schizophrenia (Chowandri 2002; Mirnics *et al.* 2001). Decreased expression of RGS4 was previously reported in several brain regions such as the prefrontal cortex, motor cortex and visual cortex (Mirnics *et al.* 2001) as well as in the superior temporal gyrus (Bowden *et al.* 2007). The inhibitory influence on RGS4 expression was also demonstrated in an animal phencyclidine (PCP) model of schizophrenia (Gu *et al.* 2007).

The functional role of RGS4 in the brain has only been partially explained. RGS4 belongs to the regulator of G-protein signaling protein (RGS) family. These proteins are GTPase activators and negatively modulate G proteins. Thus, they shorten the duration of intracellular signaling of many G-protein coupled receptors, such as dopamine, μ-opioid, metabotropic glutamate and muscarinic receptors (Ross & Wilkie 2000), many of which are implicated in schizophrenia. Various stimuli have been shown to regulate the expression of RGS genes (Burchett *et al.* 1998), and the rapidity of these changes indicates that the activity of RGS proteins is involved in the early neuronal response to stimulus. Alterations in RGS protein levels are expected to influence some receptor-mediated signaling cascades, but the mechanism mediating the role

of RGS4 in the signaling of different neuromodulators in cortical neurons is not clear.

Recent data implicate that RGS4 expression in the primate PFC is strategically positioned to regulate postsynaptic and also presynaptic signaling in response to synaptic and extrasynaptic G-protein coupled receptors (Paspalas *et al.* 2009). Therefore, it is postulated that RGS4 has a broad influence on multiple aspects of cellular physiology in the PFC (Paspalas *et al.* 2009).

The role of RGS4 in the pathophysiology of schizophrenia is likely due to its interaction with other important genes and pathways. RGS4 is related to cortical dopamine signaling (Lipska *et al.* 2006), and changes in dopamine signaling have been linked to altered expression of RGS4 mRNA in animal studies (Schwendt *et al.* 2006; Taymans *et al.* 2003). Furthermore, inhibition of RGS4 function by a specific antibody has been shown to potentiate serotonergic $5HT_{1A}$ receptor-mediated regulation of NMDA receptor channels in the PFC, while RGS4 overexpression inhibited this response (Gu *et al.* 2007). Due to these links with important neurotransmitter pathways, RGS4 is a considered to be a functional candidate gene for schizophrenia. However, a clear relationship between RGS4 and these other gene products has not yet been established. The loss of RGS4 function might lead to hyperstimulation of certain signaling pathways, consistent with the primary action of RGS proteins in increasing GTPase activity to suppress G-protein signaling (De Vries *et al.* 2000). Therefore, we examined the association of RGS4 gene downregulation with the expression of other genes to elucidate the role of this gene in the pathophysiological process of schizophrenia. We have silenced expression of RGS4 *in vitro* using siRNA with the goal to assess the influence of RGS4 gene alteration on the transcription profile *via* whole transcriptome profiling of a human neuroblastoma cell line.

METHODS

The original idea was to attempt culturing primary neuronal cells (ATCC; CRL-10442 and CRL-10742), as the most relevant substrate for the ensuing in vitro siRNA silencing experiments. Unfortunately, since we have repeatedly failed at cultivation of this type of cells (in all cases premature senescence and cell death in vitro), we decided to carry out the experiments using an immortal neuronal cell line. Thus, as a model system, the human neuroblastoma cell line BE (2)-C was used (ATCC; CRL-2268). To silence human RGS4, we designed four siRNAs covering different parts of the RGS4 coding sequence. Retroviral vector pSilencer 5.1-H1 Retro (Ambion, UK) was used to ensure

stable RGS4 silencing. Prepared constructs were transformed into *E. coli* cells by the heat shock method. Phenol-chloroform extraction was used to isolate plasmids for BE(2)-C cell line transfection. Verification of all siRNA Retro constructs designed for transfection was performed by sequencing. Scrambled siRNA cloned in the pSilencer 5.1-H1 Retro vector (Ambion, UK) was used as the background control. Transfection of the BE(2)-C cell line was carried out using FuGene Reagent (Roche, Germany) according to the recommendations of the manufacturer. Cell lines were cultivated in DMEM (Sigma, Germany) supplemented with 10% FCS (Gibco, UK) and glutamine (Sigma, Germany), at 37°C, in the presence of 5% CO2. Cell lines were grown in doublets in polystyrene-treated Petri dishes (TPP, Austria). Two days before harvest for the microarray experiments, the cells were seeded at the density of 100,000/ml. Upon harvest, the cells were washed in 1x PBS (Sigma, Germany) and directly lysed in TriZol (Sigma, Germany). Cleared lysates were used for RNA isolation, according to the instructions of the manufacturer. 1 microgram of total RNA was used for the microarray analyses, according to the manufacturer´s protocol.

Eleven stable cell lines expressing different amounts of siRNA against human RGS4 were prepared. The expression of human RGS4 was assessed by quantitative Real-Time PCR. β_2-microglobulin was used as the housekeeping gene. To study the effects of differential RGS4 expression levels in the model cell line BE (2)-C, the GeneChip® Human Exon 1.0 ST array (Affymetrix, USA) was used. The subsequent data analysis and detection of differentially expressed genes was done using a Bioconductor and R project software (http://www.bioconductor.org, http://www.r-project.org).

Microarray raw data were quartile normalized and summarized on the transcript level using the Expression Console and the default Annotation provided by Affymetrix. Normalized data were imported into R project software; quality control of the data and identification of differentially expressed transcripts among individual BE (2)-C cell lines with different RGS4 expression levels were carried out using software for statistical computing deposited at the Bioconductor repository. For quality assessment and quality control, we selected transcripts which showed high expression variability across all arrays. The criterion used was a minimal inter-quartile region (IQR) of 0.5 in log2 scale. We have calculated Euclidean distance matrix for all samples based on the filtered dataset. The samples were hierarchically clustered (using average linkage) to identify possible outliers. The same hierarchical dendrogram was used to define subgroups of BE (2)-C RGS4 mutants displaying similar expression profiles that translated into identifiable biological distinctions between

individual BE (2)-C clusters. Only genes expressed in at least one group were used for further analyses (genes with expression higher than the median of negative controls). Filtered data were tested for differences in expression using the moderated *t*-test in the Limma package from the Bioconductor repository, taking advantage of empirical Bayes moderation especially in those comparisons where the compared groups had only a few replicates. Multiple testing corrections were performed using the Benjamini & Hochberg method.

RESULTS

The resulting eleven stable cell lines demonstrated different levels of RGS4 gene expression. RGS4 expression varied from 3.1% to 123% as compared to the parental cell line. Two cell lines with minimal changes were used in the array as background (line 4, 86.7%; line 8, 123%). A further nine lines with downregulated expression were seen (lines: 1, 3.1%; 2, 25.8%; 3, 8.7%; 5, 37.9%; 6, 62.3%; 7, 41.9%; 9, 22.3%; 10, 25.8%; 11, 69.4%). In one experiment, an aberrant overexpression of genes induced by siRNA was observed. This observation represents a unique, nevertheless existing phenomenon, which is caused by aberrant interaction of some siRNAs with their target sequence. In further analyses, we evaluated whole genome transcription profiles only in cell lines with downregulated RGS4.

The cell lines with downregulated RGS4 expression showed 67 differentially expressed genes which fulfill standard criteria for RGS4 silencing. Of the 67 differentially expressed genes, 30 were underexpressed and 37 overexpressed, as compared with the parental cell line (Table 1).

When clustered using DAVID Bioinformatics Resources (Huang *et al.* 2009), we identified two functional groups of genes: the first group which contained the genes for histones (five genes: all were upregulated). The second group consisted of genes encoding for transcription factors (TF) (7 genes: four upregulated and three downregulated).

The last group of differentially expressed transcripts was heterogeneous and included some genes which have been previously associated with the pathophysiology of schizophrenia. We found three genes, overexpressed in silenced lines, which belong to this group: metallothionein (MT2A), ribonuclease A (RNASE1) and G-protein coupled receptor 50 (GPR50). Conversely, a further G-protein coupled receptor (GPR64) was underexpressed in our samples. We also identified the influence of RGS4 silencing on the expression of two other genes, CD99 and abnormal spindle-like, microencephaly-associated

Tab. 1. Genes with changed expression in the RGS4-silenced BE (2)-C cell lines.

Gene symbol	Chromosomal region	Annotation	logFC	adj. *p*-val
LRP1	12q13-q14	Low density lipoprotein-related protein 1	–2.14	0.001
LGR5	12q22-q23	Leucine-rich repeat-containing G protein-coupled receptor 5	–2.44	0.026
VEGFA	6p12	Vascular endothelial growth factor A	–2.44	0.001
CCPG1	15q21.1	Cell cycle progression 1	–2.57	0.001
LTB4DH	9q31.3	(PTGR1) Prostaglandin reductase 1	–2.60	0.001
EGR1***	5q31.1	Early growth response 1	–2.70	0.001
CRH	8q13	Corticotropin releasing hormone	–2.72	0.001
TAGLN2	1q21-q25	Transgelin 2	–2.74	0.001
LUM	12q21.3-q22	Lumican	–2.74	0.001
ATF5 ***	19q13.3	Activating transcription factor 5	–2.79	0.001
INHBE	12q13.3	Inhibin, beta E	–2.85	0.001
TRIB3	20p13-p12.2	Tribbles homolog 3	–2.89	0.001
KCNG1	20q13	Potassium volatage-gated chanel, subfamily G, member 1	–2.95	0.001
P4HA2	5q31	Prolyl 4-hydroxylase, alpha polypeptide II	–2.96	0.002
ARHGEF2	1q21-q22	Rho/rac guanine nucleotide exchange factor	–2.97	0.001
DDIT3 ***	12q13.1-q13.2	DNA-damage-inducible transcript 3	–3.01	0.001
CRABP2	1q21.3	Cellular retinoic acid binding protein 2	–3.03	0.001
TRPA1	8q13	Transient receptor potential cation channel, subfamily A, member 1	–3.14	0.001
SLC7A11•	4q28-q32	Cationic amino acid transporter, member 11	–3.16	0.001
ASS1	9q34.1	Argininosuccinate synthetase 1	–3.18	0.001
CD99‡	Xp22.32	CD99 protein	–3.19	0.002
CD99	Yp11.3	CD99 protein	–3.19	0.002
GDF15	19p13.11	Growth differentiation factor 15	–3.23	0.001
ARG2	14q24.1-q24.3	Arginase, type II	–3.24	0.001
DDIT4	10pter-q26.12	DNA-damage-inducible transcript 4	–3.29	0.007
LOC340274	7		–3.38	0.008
STC2	5q35.1	Stanniocalcin 2	–3.40	0.001
C1orf51	1q21.2	Chromosome 1 open reading frame 51	–3.65	0.999
SERPINE2	2q33-q35	Serpin peptidase inhibitor, clade E, member 2	–3.67	0.001
GPR64 †	Xp22.13	G protein-coupled receptor 64	–3.83	0.001
CHAC1	15q15.1	ChaC, cation transport regulator homolog 1	–3.83	0.001

** Histone group; *** Transcription factors; †Reported association with SCZ; ‡ Association with SCZ not confirmed previously;
• Genes products which have functional potential for SCZ but association with the disorder has not been previously detected. Logarithm of fold change (logFC), positive values represent upregulation, negative values downregulation, adjusted *p*-value (adj. *p*-val).

Tab. 1. Continue from previous page

Gene symbol	Chromosomal region	Annotation	logFC	adj. *p*-val
TM6SF1	15q24-q26	Transmembrane 6 superfamily member 1	–4.00	0.001
CENPI	Xq22.1	Centromere protein I	1.95	0.001
HIST1H2BH **	6p21. 3	Histone cluster 1, H2bh	1.96	0.003
GINS2	16q24.1	GINS complex subunit 2	1.96	0.001
GPR50 †	Xq28	G protein-coupled receptor 50	1.97	0.207
ABCA12	2q34	ATP-binding cassette, sub-family A, member 12	1.97	0.001
SPAG5	17q11.2	Sperm associated antigen 5	1.98	0.001
SNAI2 ***	8q11	Snail homolog 2	1.99	0.001
DLG7	14q22.3	Discs, large (Drosophila) homolog-associated protein 5	1.99	0.001
HIST2H2AB **	1q21	Histone cluster 2, H2ab	2.01	0.002
POLE2	14q21-q22	Polymerase (DNA directed), epsilon 2 (p59 subunit)	2.01	0.001
KIF18A	1q14.1	Kinesin family member 18A	2.03	0.001
ASPM ‡	1q31	Abnormal Spindle-like, microencephaly-associated	2.03	0.002
HIST1H2BF **	6p21.3	Histone cluster 1, H2bf	2.04	0.001
NMU	4q12	Neuromedin U	2.06	0.038
PARVA	11p15.3	Parvin alpha	2.07	0.001
RNASE1†	14q11.2	Ribonuclease, RNase A family, 1	2.10	0.999
PAC1	7p14	Adenylate cyclase-activating polypeptide 1Receptor typ 1	2.11	0.006
SHCBP1	16q11.2	SHC SH2-domain binding protein 1	2.11	0.001
FAM111B	11q12.1	Family with sequence similarity 111, member B	2.15	0.001
TYMS	18p11.32	Thymidylate synthetase	2.17	0.001
S100A4 •	1q21	S100 calcium binding protein A4	2.19	0.001
C15orf42	15q26.1	Chromosome 15 open reading frame 42	2.24	0.001
MAGEC2	Xq27	Melanoma antigen family C, 2	2.27	0.329
CAB39L •	13q14.2	Calcium binding protein 39-like	2.28	0.032
MT2A †	16q13	Methallothionein	2.28	0.001
ID2 ***	2p25	Inhibitor of DNA binding 2	2.45	0.002
SCGN	6p22.3-p22. 1	Secretagogin, EF-hand calcium binding protein	2.53	0.001
GCA	2q24. 2	Grancalcin, EF-hand calcium binding protein	2.54	0.001
HIST1H2BM **	6p22-p21.3	Histone cluster 1, H2bm	2.56	0.001
HIST1H2AB **	6p21.3	Histone cluster 1, H2ab	2.59	0.001

** Histone group; *** Transcription factors; †Reported association with SCZ; ‡ Association with SCZ not confirmed previously;
• Genes products which have functional potential for SCZ but association with the disorder has not been previously detected. Logarithm of fold change (logFC), positive values represent upregulation, negative values downregulation, adjusted *p*-value (adj. *p*-val).

Tab. 1. Continue from previous page

Gene symbol	Chromosomal region	Annotation	logFC	adj. *p*-val
ID1 ***	20q11	Inhibitor of DNA binding 1	2.83	0.002
TMSL8	Xq21.33-q22.3	Thymosin beta 15a	2.95	0.001
GNG11	7q21	Guanine nucleotide binding protein, gamma 11	3.03	0.012
ID3 ***	1p36.13-p36.12	Inhibitor of DNA binding 3	3.08	0.001
DGKK	Xp11.22	Diacylglycerol kinase, kappa	3.52	0.001
MSN	Xq11.2-q12	Moesin	4.70	0.001

** Histone group; *** Transcription factors; †Reported association with SCZ; ‡ Association with SCZ not confirmed previously;
• Genes products which have functional potential for SCZ but association with the disorder has not been previously detected. Logarithm of fold change (logFC), positive values represent upregulation, negative values downregulation, adjusted *p*-value (adj. *p*-val).

gene (ASPM), which have not been associated with schizophrenia in previous studies (Table 1). Indeed, copy number of the gene for CD99 protein was studied in schizophrenic patients with negative results (Ross *et al.* 2003), and a single-nucleotide polymorphisms (SNPs) for the ASPM gene were not found to be in association with schizophrenia (Rivero *et al.* 2006). Other genes in this group have protein products with functions potentially related to schizophrenia (Table 1): solute carrier family 7 member 11 (SLC7A11), calcium binding protein 39-like (CAB39L) and S100 calcium binding protein A4 (S100A4).

DISCUSSION

The silencing of RGS4 by siRNA changed expression of 67 genes, which could be divided into two functional groups (histone genes and transcription factors) and a heterogeneous group of genes. Interestingly, we did not detect changes in expression of any of the genes directly connect with serotonin, dopamine, glutamate or other receptors associated with schizophrenia.

Although this study was carried out on an immortal neuroblastoma cell line and not on primary neurons (which would approximate better for the authentic situation *in vivo*), the results might shade some light on the complex issue of the pathogenesis of schizophrenia.

The upregulation of H2A/B histone protein expression detected in our study corresponds with a possible role of these proteins in schizophrenia. Histone modifications represent one of the epigenetic mechanisms connected with pathology of the prefrontal cortex in schizophrenia (Akbarian *et al.* 2005).

Post-translational modifications (PTMs) like acetylation, phosphorylation, sumoylation and ubiquitinylation connected with H2A/B are known to influence gene transcription (Berger *et al.* 2007). We speculate that the overexpression of H2A/B histone in RGS4-silenced cells may enhance these PTMs.

The second group of genes influenced by siRNA for RGS4 includes the genes for TF traditionally associated with cancer (Janoueix-Lerosey *et al.* 2010). Therefore, the changes in TF expression observed in our experiment might be a side effect of the neuroblastoma cell line used. However, the downregulation of the gene for early growth response 1 (EGR1) in our cell lines with silenced RGS4 belongs to the family of early-immediate genes (IEG) which may play an important role in learning and memory (Bozon *et al.* 2003). Downregulation of RGS4 and EGR1 has been reported in mice following the deletion of brain-derived neurotrophic factor (BDNF) (Glorioso *et al.* 2006). In this context, we postulate that there might be an interaction between expression of this IEG and RGS4. Furthermore, in RGS4-silenced lines, downregulation of the gene for Activating Transcription Factor 5 (ATF5), which binds on the protein level to disrupted in schizophrenia 1 (DISCI1), might suggest some importance in schizophrenia pathogenesis. We could not determine any clear connection with the pathophysiology of schizophrenia for the other TF genes in our study.

The third heterogeneous group included some genes with a reported association with schizophrenia. We detected overexpression of the metallothionein (MT2A) gene in RGS4-silenced cell lines. The physiological function of this protein in the CNS involves neuroprotection and regeneration (West *et al.* 2008), so upregulation of MT2A might be a compensatory reaction to a lack of RGS4. Upregulation of the gene for MT2A in schizophrenia has also been reported in previous postmortem studies in the prefrontal (Arion *et al.* 2007) and temporal cortex (Aston *et al.* 2004).

Following RGS4 silencing, we also found overexpression of the RNASE1 gene. However, in two previous studies, downregulated expression of this gene was found by postmortem analysis in the temporal cortex of patients with schizophrenia (Aston *et al.* 2004) and in the dorsolateral prefrontal cortex of elderly patients with chronic schizophrenia (Hakak *et al.* 2001).

Additionally, overexpression of the gene for GPR50 has been reported in connection with schizophrenia and bipolar disorder (Kato 2007), although an association with SNPs for this gene was detected only in female patients. One previous study has demonstrated a strong association between RGS4 and several G-protein coupled receptors (GPCRs) that are central participants in

normal and pathologically-altered neuromodulation of membrane excitability (Jaén&Doupnik 2006). However, although GPR50 is a melatonin-related receptor, it does not bind melatonin and its endogenous ligand is currently unknown. This receptor has been shown to behave as an antagonist of the melatonin receptor MT1 (Levoye *et al.* 2006). Inhibition of MT1 function is mediated by heterodimerization of MT1 with GPR50, and the relative propensity of the formation of this heterodimer compared to GPR50 homodimers depends on the relative expression of both receptors. It is important that when MT1 is incorporated into GPR50/MT1 heterodimers, it loses its ability to bind melatonin receptor specific agonists (Levoye *et al.* 2006).

Downregulation of RGS4 in our cell lines was connected with upregulation of adenylate cyclase-activating polypeptide 1 receptor 1 (ADCPYAP1R1; PAC1). PAC1 is a G-protein-linked receptor and its ligand PACAP is a neuropeptide with neurotransmission-modulating activity. Both this receptor and its ligand are reported as schizophrenia candidate genes (Hashimoto *et al.* 2007). In a mouse model of schizophrenia induced by chronic PCP treatment, increased expression of PAC1 mRNA was seen in the frontal cortex and in the hippocampus (Hashimoto *et al.* 2007). We found that siRNA against RGS4 modifies the expression of the genes for SLC7A11, CAB39L, S100A4, although their role in schizophrenia still needs to be clarified. The two overexpressed genes, S100A4 and CAB39L, code for calcium binding proteins which influence various cellular responses along the calcium signal-transduction pathway. The related protein S100B has recently been shown to be a marker of systemic inflammation (Sen & Belli 2009). It has been also reported that this protein is elevated in the serum of schizophrenic patients (Schroeter *et al.* 2009). SLC7A11 was underexpressed in the RGS4-silenced cell lines. Its protein product is a member of the amino acid transport system specific for cysteine and glutamate. Thus, SLC7A11 might be implicated in glutamate pathway disorders which have been reported in schizophrenia pathophysiology (Carter 2006).

CONCLUSIONS

We assessed three subgroups of genes which might represent a part of the pathophysiological background of schizophrenia: histone genes, which were overexpressed in RGS4-silenced cells lines and may play a role in epigenetic mechanisms of schizophrenia pathophysiology; TF genes which are connected with other schizophrenia-relevant genes like BDNF and DISCI1; and a heterogeneous group which contains G-proteins and calcium binding proteins.

Subsequent experiments would elucidate the role of these genes in the complex pathogenesis of schizophrenia.

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REFERENCE

1 Akbarian S, Ruehl MG, Bliven E, Luiz LA, Peranelli AC, Baker SP, *et al.* (2005) Chromatin alterations associated with down-regulated metabolic gene expression in the prefrontal cortex of subjects with schizophrenia. Arch Gen Psychiatry. **62**(8): 829–40.
2 Arion D, Unger T, Lewis DA, Levitt P, Mirnics K (2007) Molecular evidence for Increased Expression of Genes Related to immune and Chaperone Function in the Prefrontal cortex in Schizophrenia. Biol Psychiatry. **62**: 711–21.
3 Aston C, Jiang L, Sokolov PB (2004) Microarray analysis of postmortem temporal cortex from patients with schizophrenia. J Neurosci Res. **77**: 858–866.
4 Berger SL (2007) The complex language of chromatin regulation during transcription. Nature. **447**: 407–412.
5 Bowden NA, Scott RJ, Tooney PA (2007) Altered expression of regulator of G-protein signalling 4 (RGS4) mRNA in the superior temporal gyrus in schizophrenia. Schizophr Res. **89**: 165–168.
6 Bozon B, Kelly A, Josselyn SA, Silva AJ, Davis S, Laroche S (2003) MAPK, CREB and zif268 are all required for the consolidation of recognition memory. Phil Trans R Soc B. **358**: 805–814.
7 Burchett SA, Volk ML, Bannon MJ, Granneman JG (1998) Regulators of G proteins signaling: rapid changes in mRNA abundance in response to amphetamine. J Neurochem. **70**: 2216–2219.
8 Carter CJ (2006) Schizophrenia susceptibility genes converge on interlinked pathways related to glutamatergic transmission and long-term potentiation, oxidative stress and oligodendrocyte viability. Schizoph Res. **86**: 1–14.
9 Chowadri KV (2002) Association and linkage analyses of RGS4 polymorphisms in schizophrenia. Human Molecular Genetics. **11**: 1373–80.
10 Chowdari KV, Bamne M, Joel W, Talkowski ME, Mirnics K, Levitt P, *et al.* (2008) Linkage Disequilibrium Patterns and Functional Analysis of RGS4 Polymorphisms in Relation to Schizophrenia. Schizophr Bull. **34**: 118– 26.
11 De Vries L, Zheng B, Fischer T, Elenko E, Farquhar MG (2000) The regulator of G protein signaling family. Annu Rev Pharmacol Toxicol. **40**: 235–271.
12 Erdley HA, Lahti RA, Lopez MB, Myers CS, Roberts RC, Tamminga CA., *et al.* (2004) Regional expression of RGS4 mRNA in human brain. Eur J Neurosci. **19**: 3125–3128.
13 Glorioso C, Sabatini M, Unger T, Hashimoto T, Monteggia LM, Lewis DA *et al.* (2006) Specificity and timing of neocortical transcriptome changes in response to BDNF gene ablation during embryogenesis or adulthood. Mol Psychiatry. **11**: 633–648.
14 Gu Z, Jiang Q, Yan Z (2007) RGS4 modulates serotonin signaling in prefrontal cortex and links to serotonin dysfunction in a rat model of schizophrenia . Mol Pharmacol. **71**(4): 1030–1039.
15 Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, *et al.* (2001) Genome–wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci USA. **98**: 4746–4751.

16 Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, *et al.* (2007) Pituitary adenyle cyclase-activating polypeptide is associated with schizophrenia. Molecular Psychiatry. **12**: 10026–32.
17 Huang DW, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. **4**(1): 44–57.
18 Jae´n C, Doupnik CA (2006) RGS3 and RGS4 Differentially Associate with G Protein-coupled Receptor-Kir3 Channel Signaling Complexes Revealing Two Modes of RGS Modulation. Journal of Biological Chemistry. **281**: 34549–34560.
19 Janoueix-Lerosey I, Schleiermacher G, Delattre O (2010) Molecular pathogenesis of peripheral neuroblastic tumors. Oncogene. **29**(11): 1566–79.
20 Kato T (2007) Molecular genetics of bipolar disorder and depression. Psych and Clin Neurosciences. **61**: 3–19.
21 Levoye A, Dam J, Ayoub MA, Gulliaume JL, Coturier C, Delagrange P *et al.* (2006) The ophram GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. EMBO J. **25**: 3012–3023.
22 Lipska BK, Mitkus S, Caruso M, Hyde TM, Chen J, Vakkalanka R, *et al.* (2006) RGS4 mRNA expression in postmortem human cortex is associated with COMT val158met genotype and COMT enzyme activity. Hum Mol Genet. **15**: 2804–2812.
23 Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P (2001) Disease-specific changes in regulator of G-protein signalling 4 (RGS4) expression in schizophrenia. Molecular Psychiatry. **6**: 293–301.
24 Paspalas CD, Selemon LD, Arnsten AFT (2009) Mapping the regulátor of G protein signaling 4(RGS4): Presynaptic and postsynaptic substrates for neuroregulation in prefrontal cortex. Cerebral Cortex. **19**: 2145–2155.
25 Rivero O, Sanjuán J, Maltó MD, Aquilar EJ, Gonzalez JC, deFrutos R, *et al.* (2006) The microencephaly ASPM gene and schizophrenia: A preliminary study, Schizophr Res. **84**: 427–429.
26 Ross EM, Wilkie TM (2000) GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem. **69**: 795–827.
27 Ross NLJ, Mavrogiannis LA, Sargent CA, Knight SJL, Wadekar R, DeLisi LE, *et al.* (2003) Quantification of X-Y homologous genes in patients with schizophrenia by multiplex polymerase chain reaction. Psychiatr Genet. **13**: 115–119.
28 Schroeter ML, Abdul-Khaliq H, Krebs M, Diefenbacher A, Blasig IE (2009) Neuron-specific enolase is unaltered whereas S100B is elevated in serum of patients with schizophrenia – Original research and meta-analysis. Psy Res. **167**: 66–72.
29 Schwendt M, Gold SJ, McGinty JF (2006) Acute amphetamine down-regulates RGS4 mRNA and protein expression in rat forebrain: distinct roles of D and D dopamine receptors. J Neurochem. **96**: 1606–1615.
30 Sen A, Belli A (2009) S100B in neuropathologic states: The CRP of the Brain? J Neurosci Res. **85**: 1373–1380.
31 Taymans JM, Leysen JE, Langlois X. (2003) Striatal gene expression of RGS2 and RGS4 is specifically mediated by dopamine D1 and D2 receptors: clues for RGS2 and RGS4 functions. J Neurochem. **84**: 1118–1127.
32 West AK, Hidalgo J, Eddins D, Levin ED. Aschner M (2008) Metallothionein in the central nervous system: Roles in protection, regeneration and cognition. Neurotoxicology. **29**: 489–503.