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Genome-wide expression analysis of peripheral blood identifies candidate biomarkers for schizophrenia

Running title: Candidate biomarkers for schizophrenia

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Abstract

The aim of this study was to analyze gene expression in blood of patients with newly-diagnosed schizophrenia during their first psychotic episode and subsequent remission. Whole blood samples were obtained from 32 untreated patients presenting with their first psychotic episode suggestive of schizophrenia and 32 age- and gender-matched controls. Using Affymetrix micoarrays, we identified significantly altered expression of 180 gene probes in psychotic patients compared to controls. A subset of 4 significantly changed genes was further confirmed with QRT PCR. The following genes were significantly altered in patients: glucose transporter, SLC2A3 ($p<0.001$) and actin assembly factor DAAM2 ($p<0.001$) were increased, whereas translation, zinc metallopeptidase, neurolysin 1 and myosin C were significantly decreased ($p<0.05$). Expression of these candidate markers was also analyzed in a longitudinal study (12-24 months) in 12 patients who achieved full remission. Interestingly, expression of DAAM2 returned to control levels in patients who were in remission after their first psychotic episode, suggesting that its expression correlates with diseases progression and/or response to treatment. In summary, we identified changes of gene expression from peripheral blood which might help discriminate patients with schizophrenia from controls. While these results are promising, especially for DAAM2 whose polymorphic variants have been found significantly associated with schizophrenia, it will be important to analyze larger cohorts of patients in order to firmly establish changes in gene expression as blood markers of schizophrenia.

Key words: schizophrenia, gene expression, biomarkers, blood, DAAM2

1. INTRODUCTION

Schizophrenia is one of the most disabling psychiatric illnesses, affecting about 1% of population, with no significant differences in its prevalence among countries (Sadock & Sadock, 2003). Although the term schizophrenia is used to describe a relatively heterogeneous group of clinical presentations, in majority of cases, schizophrenia is a chronic illness with a recurrent course, characterized by alternating periods of acute psychotic illness and their remission. A prodromal stage which may last for several years prior to presentation of acute psychotic symptoms usually precedes the first acute psychosis. Duration of at least six months of the first episode of psychosis is highly suggestive of schizophrenia. With each new episode of acute psychosis, the patient condition deteriorates further, until a stage with predominately negative and cognitive symptoms is reached.

The pathogenesis of this illness is still largely unknown and the diagnosis of schizophrenia is based on its clinical presentation. In both clinical and research settings, DSM-IV criteria are usually applied (APA, 1994). The severity of symptoms is usually determined using standardized measurement tools, such as positive and negative syndrome scale (PANSS) (Kay et al. 1987). Adequate treatment response of an acute episode is characterized by a 50% reduction of the severity of acute symptoms after 6 weeks (early treatment response) or 12 weeks of treatment (late treatment response) (Kane et al. 2003). Strict remission criteria were proposed by Andreasen et al. (2005).

To objectify the diagnosis and course of treatment of this illness, an effort has been made to identify a biological correlate of schizophrenia (Sadock & Sadock, 2003). For example, the search for biomarkers revealed neuropathological (Shapiro, 1993;

Farrow et al. 2005) and neurophysiologic (Callicott et al. 1999) abnormalities, as well as alterations in neurotransmitter function (Stahl, 2000), neurotrophic factors (Parikh et al. 2003) and gene expression in prefrontal cortex (Mirnics et al. 2001).

For clinical purpose, a biomarker which might be detected by non- invasive methods would be desirable. To this end, several authors identified possible biomarkers for schizophrenia from peripheral blood, including neurotransmitter metabolite levels, stress hormones (Sadock & Sadock, 2003), markers of immune response (Gladkevich et al. 2004) and fatty acids (Fenton et al. 2000). Recently, it has been suggested that gene expression analysis of peripheral blood microarrays might become a potentially useful tool in detecting markers of presence and progression of a variety of neuropsychiatric diseases including schizophrenia (Gladkevich et al. 2004; Middleton et al. 2005).

The focus of this study has been to examine gene expression changes during the first psychotic episode and subsequent remission in newly-diagnosed patients with schizophrenia. This study design allows for monitoring of changes in gene expression in relation to disease progression and response to treatment.

2. SUBJECTS AND METHODS

2. 1. Subjects

We analyzed 32 previously untreated patients presenting with their first psychotic episode suggestive of schizophrenia, admitted at Zagreb University Hospital Center in the period from 2004 to 2007. All patients were over 18 years, Caucasian, met criteria for a diagnosis for schizophrenia following DSM-IV guidelines (2). The inclusive

criteria were as follows: (1) Acute psychosis defined by the score of least 80 on the positive and negative syndrome scale (PANSS) (Kay et al. 1987) and at least graded by moderate severity on at least two positive symptoms (2) DSM - IV criteria met for schizophrenia; (3) no other neurological diseases, mental disorders, drug and alcohol abuse and eating disorders, no other serious somatic illness, including acute infections; (4) normal blood counts; (5) positive family history of schizophrenia.

The severity of illness was assessed using the PANSS scale, by two different psychiatrists. Global functioning was assessed using Global Assessment of Functioning (GAF) Scale (DSM-IV). All relevant sociodemographic, including ethnic and geographical background, and clinical data were assessed during the clinical interview (Table 1).

Controls were all age and sex matched healthy subjects of the same ethnic and geographical background as patients, and were matched also in respect to blood count composition.

2. 2. Study protocol

The study was conducted in two steps.

After a careful selection of patients who fulfilled all above mentioned inclusion and exclusion criteria, in the first study point whole blood samples were obtained from all patients prior to the administration of medication. At the same time whole blood samples were obtained from sex- and age- matched controls. After the blood samples were obtained, all patients received a second generation antipsychotic (SGA) according to the standard clinical practice.

The next step of the study design involved the follow up of all subjects for at least one year period, during which all subjects continued the pharmacological treatment. The follow-up consisted of regular outpatient visits which included psychiatric interviews and assessments of the severity of illness using PANSS and GAF. Full remission was determined using strict criteria (Andreasen et al. 2005). Additionally, only those who returned to the previous level of functioning (work, school) assessed using GAF were regarded as remitted. Out of 32 patients, only 14 patients fulfilled the requirements for full remission and returned to the previous level of functioning, and were included in this second part of the study, but two patients refused to participate further in the study. The rest of patients achieved only partial remission even after 2-year follow up (N=15), were lost from the follow up soon after hospital discharge (N=2) whereas one patient committed suicide.

All participants gave informed consent for participation in the study, and they were given explanations and answers about the procedure of the study. The study protocol was approved by the Hospital Ethical Committee of the Zagreb University Hospital Centre.

2. 3. RNA expression analysis

2.3.1. Isolation of the blood samples

In the first step, blood samples from a total of 32 patients with schizophrenia (8 male and 24 female) and 32 sex and age matched controls were obtained. In the second step, blood samples from 12 patients (1 male and 11 female) who achieved full

remission after a 12 to 24 month-period were obtained. Blood specimens of cases and controls were collected and assayed in parallel.

Total RNA from blood was extracted by using PAXgene blood RNA kit (Qiagen). All samples were treated with the RNase-free DNase set (Qiagen). The quality of total RNA was analyzed by using the RNA 6000 Nano LabChip on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Microarray analysis was performed using U133A GeneChips (Affymetrix) with 22,283 30-mer probes (approximately 11000 genes). Total RNA was processed using Affymetrix protocol: 4 μg of high quality RNA was reverse-transcribed, purified (Invitrogen, Qiagen) and than used as a template for in-vitro transcription by using T7 MEGA script reagents and biotin-11-UTP. Biotin labeled RNA was purified with the RNeasy kit (Qiagen), hybridized to chips, fluorescently tagged and scanned. The regular quality measures for Affymetrix GeneChip were used.

2.2. Quantitative RT-PCR analysis

Reverse transcription was performed using total RNA isolated from blood, processed with the SuperScript II first strand synthesis for RT-PCR following the manufacturers protocol (Invitrogen). For detection in quantitative RT-PCR SYBR green was used. Primers were as follows: catenin (cadherin-associated protein) beta 1 (CTNNB1) F 5' ATGCGTTCTCCTCAGATGGT and R 5' TGGGAAAGGTTATGCAAGGT; RING1 and YY1 binding protein (RYBP) F 5' CGCTCCTCATCGACATCCT and 5' R AGCCCTTGTCTGTGCTCTCT; ring finger protein 10 (RFN10) F 5' GGCAAGTACCCAGAAGTCCA and R 5' GCAGAATCAGAGGAGCAGGT; glucose transporter, SLC2A3 F 5' TGCTTAGGAGAGACCGAGTGA and R 5'

ATATCAGAACCCAAGGGAGGA; neurolysin 1 (NLN-1) F 5' GGCTGAACTTGGTGCTCTTC and R 5' TAGTTTGGCCACCTTGGTTC; myosin C (MYO1C) F 5' ATCCCATTATGAGCCAGTGC and R 5' CATCATTGGGTTTGATGCAG; actin assembly factor (DAAM2) F 5' GCTGCCAAAGTCAACCTAGC and R 5' CTTGTCCCTGGCCTCATTTA; ß-actin (ACTB) F 5' GGACTTCGAGCAAGAGATGG and R 5' AGCACTGTGTTGGCGTACAG. ß-actin was used as an internal control. We used two copies of each sample.

3. Statistical analysis

MAS (version 5.0) was used for the calculation of signal values on microarrays and ACCESS for filtering the statistically significant genes. Calculation of maximal values of expression, t-tests (two sided) and ratios of change were performed using EXCEL, Microsoft office. In the further analysis of the microarray data, only genes that fulfilled three conditions were included: 1. MAS "signal intensity" above the "target intensity" of 100 to reduce the possibility of the error due to technical noise; 2. expression ratio of average psychosis/control >1.8 or <0.6; 3. $p<0.05$ on two sided student t-test. *P* values were corrected for multiple testing using a false discovery rate approach. Affymetrix microarrays were normalized using Robust Multi-array Averaging. Analysis was performed using R version 2.3 and the Bioconductor packages affy and limma (http://www.bioconductor.org) (Gentleman et al. 2004). Where multiple probe sets were assigned to the same gene only the statistics from the probe with the greatest evidence for differential expression were retained. Affymetrix annotations (version 24) were used for probe-set-to-gene assignments.

For QRT-PCR data, the differences in the Δ Ct between the genes and ß-actin were used for t-test statistics. Differences in expression levels of each gene between patients and controls were analyzed using t-test for independent samples, two sided, whereas differences in expression levels of each gene between patients in acute psychosis and their remission were analyzed using t-test for dependent samples, two sided. The average fold change = $2^{-(\text{average } \Delta \text{ Ct})}$ was computed using the average Δ Δ Ct between patients and controls and between patients in acute psychosis and their remission. Mean standard error SEM was calculated using Δ Ct.

We used descriptive statistics for showing clinical features at baseline. The associations of expression levels of each gene (Δ Ct between the genes and ß-actin) and a variety of clinical features were studied using multivariate analysis. No changes were observed in beta-actin among various groups examined.

For all clinical and QRT-PCR data analysis EXCEL, Microsoft office and SPSS 13.0 were used. P value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Clinical features

Description of clinical features of the patients is shown in Table 1.

3.2. Microarray analysis

Using Affymetric micorarray platform we identified 180 gene probes that were significantly changed in patients with schizophrenia compared to healthy controls and

fulfilled all three requirements ($p<0.05$, expression level 100, average expression ratio >1.8 or <0.6). 115 gene probes were down-regulated, whereas 65 were upregulated compared to controls (Supplementary table). Genes with altered expression include genes from different functional groups: transcription/RNA processing, ubiquitin, lipid/glucose/protein metabolism, signal transduction, cytoskeleton and others (analyzed with GeneOntology).

3.3. QRT-PCR analysis

Out of 180 significantly altered gene probes we selected a subset of 7 potential markers for further confirmation with QRT PCR: catenin (cadherin-associated protein), beta 1 (CTNNB1), RING1 and YY1 binding protein (RYBP), ring finger protein 10 (RFN10), glucose transporter (SLC2A3), zinc metallopeptidase, neurolysin 1 (NLN-1), myosin C (MYO1C) and actin assembly factor (DAAM2). Potential genetic markers were selected based on their expression levels (MAF), significance of changes between psychosis vs. controls (p-values), expression ratios of psychosis vs. controls and their function, as reported in the literature.

In total, 32 patients and 32 age- and sex- matched controls were tested. Using this approach, the following 4 genes were validated as significantly altered in patients compared to controls: glucose transporter, SLC2A3 ($t=-4.678$, $p<0.001$) and actin assembly factor DAAM2 ($t=-3.709$, $p<0.001$) were increased, whereas zinc metallopeptidase, neurolysin 1 ($t=2.838$, $p=0.006$) and myosin C ($t=2.148$, $p=0.036$) were significantly decreased (Figure 1). It is important to note that our PCR primers for SLC2A3 cannot distinguish SLC2A3 from another glucose transporter SLC2A14. Nevertheless, our microarray datasets showed that only SLC2A3-specific probe gave

significant result, suggesting that SLC2A3 is the likely gene altered in patients. We found no significant associations of sociodemoghraphic features (including age, sex, duration of untreated psychosis and family history of psychiatric disorders) with gene expression in the additional univariate analysis (data not shown).

3.4. QRT-PCR analysis for disease progression

The second step involved testing gene expression of the 4 validated candidate markers in 12 out of 32 patients who achieved full remission during a longitudinal study (12-24 months). Interestingly, expression of DAAM2 significantly decreased in patients who were in remission after their first psychotic episode ($t=-3.007$, $p=0.012$), and returned to almost control levels (Figure 2). There were no significant changes of expression of other 3 genes in patients in their state of acute psychosis compared to their state of remission (Figure 2). While there was significant difference in the expression of neurolysin 1 in remission compared to controls ($t=-3.172$, $p=0.003$), we found no significant differences in the expression of other genes in these groups (Figure 2).

4. DISCUSSION

In this study we used microarrays to detect alterations of gene expression in blood samples from patients with acute psychosis compared to healthy controls. Using real-time PCR, we confirmed altered expression of a subset of 4 genes, of which one gene (DAAM2) returned to controls levels in patients who achieved complete clinical remission. These results suggest that expression of DAAM2 correlated with disease progression and/or response to treatment.

Interestingly, Proitsi et al. (2008) found significant associations of polymorphic variants of DAAM2 gene and schizophrenia in a family trios (two haplotypic associations) and combined family trios and case control sample (single marker association), although the authors did not confirm this finding in the case - control sample. Although their findings are inconclusive, it might suggest that DAAM2 polymorphic loci might be associated with a greater risk for schizophrenia. In light of our findings, it would be of interest to examine whether these polymorphisms are associated with altered expression of DAAM2 observed in our study. Although DAAM2 is expressed in brain tissues, and it may have a role in neuronal cell differentiation (Kida et al. 2004), it precise function remains unknown. Interestingly, we observed that alteration in expression of DAAM2 in psychotic patients returned to baseline levels upon complete clinical remission, suggesting that DAAM2 may represent *a state* marker of the disease. Alternatively, changes in DAAM2 expression may be due to treatment with the antipsychotics. Although there were no significant differences in DAAM2 expression in regard to the type of antipsychotic used (data not shown), the influence of medication on DAAM2 expression cannot be completely excluded. It would be of interest to examine expression of DAAM2 in extended

follow-up studies and include patients who discontinue the treatment after a prolonged period of full remission.

In contrast to DAAM2, NLN-1 expression in patients with schizophrenia is consistently decreased compared to controls, regardless of the state of the illness. Pollio et al. (2008) reported that the overexpression of NLN-1 was neuroprotective against toxicity of amyloid ß and also observed it in brain of humans with Alzheimer disease. Considering the neurodegeneration observed in patients with schizophrenia (Benes, 2000), it would be of interest to examine the expression of NLN-1 in blood and brain of patients in schizophrenia as well in follow up studies also involving patients with chronic illness.

Gene expression analysis of peripheral blood using microarray have become increasingly accepted as a potentially useful tool in detecting markers of presence and progression of a variety of neuropsychiatric diseases. Several previous reports found differentially expressed genes in peripheral blood of patients with schizophrenia compared to their unaffected siblings (Vawter et al. 2004; Middleton et al. 2005; Kakiuchi et al. 2008) or to controls (Glatt et al. 2005; Tsuang et al. 2005; Numata et al. 2007). The usual confounders include methodological differences in data collection, heterogeneity of clinical presentations of the illness (Numata et al. 2007) and medications used by study subjects (Colantuoni et al. 2008). In addition, differences in data analysis arise from the type of microarray assays and the methodology of analysis applied (Ludwig and Weinstein, 2005).

Newer studies tried to overcome these confounders by including patients with first episode psychosis who were drug free prior to their study enrollments (Zvara el al. 2005; Numata et al. 2007; Numata et al. 2008; Suzuki et al. 2008; Zhang et al. 2008).

However, with the exception of the recent studies, all other studies were cross sectional - blood samples were drawn from patients only in their acute psychotic state, and did not address the episodic nature of the illness.

Zhang et al. (2008), found decreased expression of neuregulin1 in the blood of patients with schizophrenia compared to controls, which increased with 4-week antipsychotic treatment. Although we observed similar but non-significant trend in our datasets (data not shown), neuregulin 1 was not selected for further validation also since its MAS "signal intensity" was below 100.

Similarly, we did not observe significant differences in the expression levels of transforming grow factor-ß receptor 2 (TGFBR2), as reported by Numata et al., (2008). They found that TGFBR2 mRNA levels in medication free patients with schizophrenia were significantly higher than those of control subjects and decreased to control levels after several week of antipsychotic treatment. However, TGFBR2 mRNA levels did not correlate with the severity of illness measured with Brief Psychiatric Rating Scale (BPRS). Suzuki et al. (2008) found that levels of very low density lipoprotein receptor (VLDLR) mRNA in drug-naive patients with schizophrenia were significantly lower than those of controls and showed significant increases with respect to baseline after six months of antipsychotic treatment, with a negative correlation between VLDLR mRNA levels and the severity of clinical symptoms, measured with BPRS. In our study, VDLDR was also not selected for QRT-PCR validation since its MAS "signal intensity" was below 100.

Although we used a study design which makes our results comparable to the study of Numata et al. (2008), Suzuki et al. (2008) and Zhang et al. (2008) (inclusion of only drug- naive patients and follow up of the patients), we took further steps to reduce the heterogeneity of the samples by selecting only patients with a severe acute episode of

psychosis, and having at least one close relative affected with schizophrenia. Also, to further confirm the correlations of potential markers with disease progression, the second blood draw was determined by the clinical presentation and not by a predefined time frame - we analyzed gene expression in blood of the same patients after they have achieved full remission following very strict criteria (Andreasen et al. 2005) and returned to their previous level of functioning.

Although we found no significant differences in gene expression of the investigated markers in association with patient gender, the predominance of the female patients especially in our validation studies might have masked possible gender differences.

Despite the fact that our results are promising it will be critically important to validate these findings in larger cohorts of patients in a follow up design in order to firmly establish changes in gene expression as blood markers of schizophrenia.

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Table 1. Clinical features of the patients (N=32) at the time of their hospital admission

Feature	*Value*	
Sex (N (%))	Male	8 (25%)
	Female	24 (75%)
Age (years+/-SD)	28.15+/-7.9	
Education (years+/-SD)	11.93+/-9.67	
Duration of untreated illness (months+/-SD)	20.61+/-23.15	
Family history of psychiatric disorders (N(%))	Schizophrenia-related in close relatives	20 (62.4%)
	Schizophrenia-related multiple members	6 (18.8%)
	Schizophrenia-related in distant relatives	3 (9.4%)
	Schizophrenia-related in distant relatives and other psyciatric disorder in close relatives (alcholol addictions)	3 (9.4%)
Positive PANSS (mean+/-SD)	32+/-5.5	
Negative PANSS (mean+/-SD)	24+/-5.5	
General PANSS (mean+/-SD)	46+/-8.9	
Total PANSS (mean+/-SD)	102+/-16.5	
GAF (mean+/-SD)	26.68+/-8	

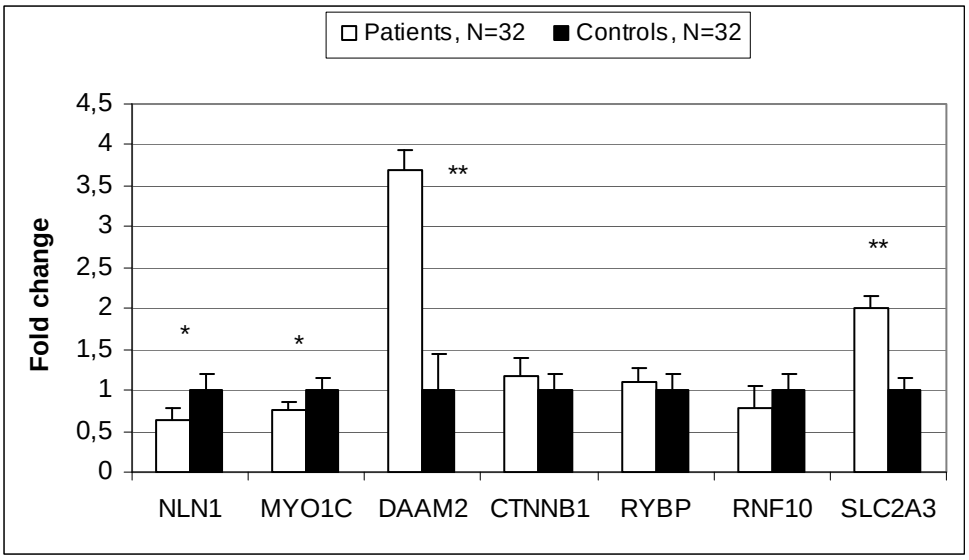

Figure 1. Fold changes in expression of 7 marker genes in 32 patients with psychosis compared to matched controls. Columns represent the average fold change = $2^{-(\text{average } \Delta Ct)}$ of expression of the genes analyzed with QRT-PCR. SEM was calculated using Δ Ct. The difference in mean Δ Ct between patients and controls was tested using t-tests for independent samples, 2-sided.

* $p<0.05$

** $p<0.001$

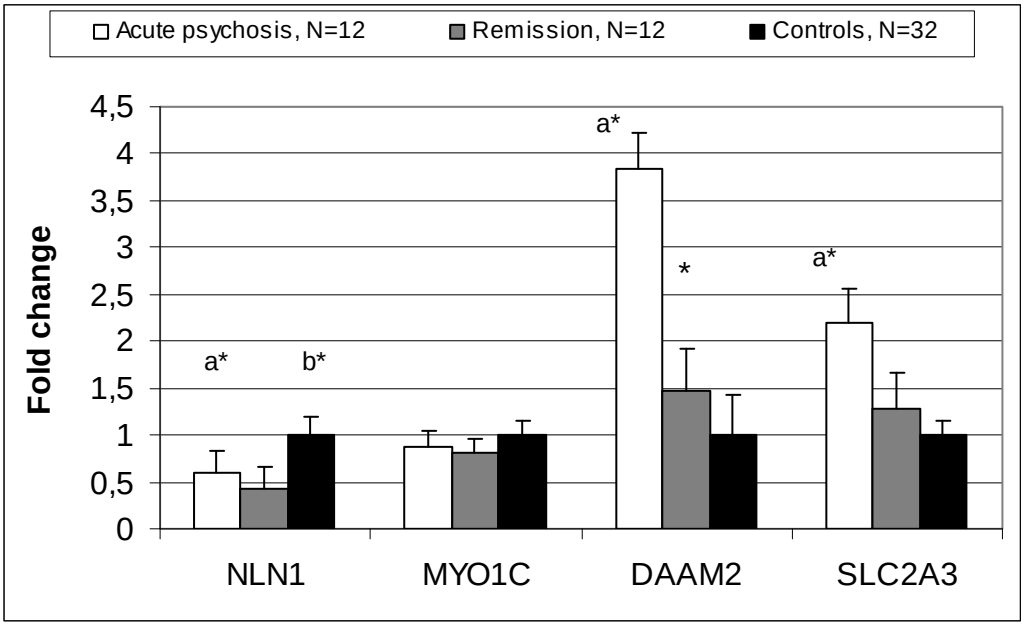

Figure 2. Fold changes in expression of 4 validated marker genes in 12 patients in the state of acute psychosis relative to controls and patients in remission relative to controls. Columns represent the average fold change = $2^{-(\text{average } \Delta \text{ Ct})}$ of expression of the 4 genes analyzed with QRT-PCR.

a* $p<0.05$, patients in acute psychosis vs. controls (t-tests for independent samples, 2-sided: NLN1: t=2.593, p=0.013; DAAM2: t=-2.671, p=0.011; SLC2A3: t=-3.559, p=0.001)

b* p=0.003, patients in remission vs. controls (t-tests for independent samples, 2-sided)

*p=0.012, patients in acute psychosis vs. remission (t-tests for dependent samples, 2-sided)

Supplementary table. Differential RNA expression levels in patients with psychosis and healthy matched control obtained with Affymetric micorarray platform. 180 gene probes are shown that were significantly changed in patients with acute psychosis compared to healthy controls ($p<0.05$, expression level 100, average expression ratio >1.8 or <0.6).

Gene	*Probe*	*Gene*	*Symbol*
Up-regulated			
	Transcription/RNA processing		
	201844_s_at	RING1 and YY1 binding protein	RYBP
	1560228_at	snail homolog 3 (Drosophila)	SNAI3
	209253_at	sorbin and SH3 domain containing 3	SORBS3
		similar to RNA binding motif protein, Y chromosome, family 2 member B	
		RNA binding motif protein, Y-linked, family 1, member A1	RBM, RBM
		RNA binding motif protein, Y-linked, family 1, member B	RBMY1A1
		RNA binding motif protein, Y-linked, family 1, member D	RBMY1B
		RNA binding motif protein, Y-linked, family 1, member E	RBMY1D
		RNA binding motif protein, Y-linked, family 1, member F	RBMY1E
		RNA binding motif protein, Y-linked, family 1, member H	RBMY1F
		RNA binding motif protein, Y-linked, family 1, member J	RBMY1H
		RNA binding motif protein, Y-linked, family 3, member A	RBMY1J
	216842_x_at	pseudogene	RBMY3AP
	1558215_s_at	upstream binding transcription factor, RNA polymerase I	UBTF
	235145_at	zinc finger and BTB domain containing 7B	ZBTB7B
	244752_at	zinc finger protein 438	ZNF438
	218222_x_at	aryl hydrocarbon receptor nuclear translocator	ARNT
	226416_at	three prime histone mRNA exonuclease 1	ERI1
	213098_at	RCD1 required for cell differentiation1 homolog (S. pombe)	RQCD1
	216925_s_at	T-cell acute lymphocitic leukemia 1	TAL1
	214263_x_at	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa	POLR2C
	Signal transduction		
	222453_at	cytochrome b reductase 1	CYBRD1
	207721_x_at	histidine triad nucleotide binding protein 1	HINT1
		similar to protein kinase, cAMP-dependent, regulatory,	LOC729623
	212555_at	type I, beta protein kinase	PRKAR1B
	224859_at	CD276 molecule	CD276
	210773_s_at	formyl peptide receptor 2	FPR2
	229395_at	syntaxin 4	STX4
	227067_x_at	Notch homolog 2 (Drosophila) N-terminal like	NOTCH2NL
	226878_at	major histocompatibility complex, class II, DO alpha	HLA-DOA
	206614_at	growth differentiation factor 5	GDF5
	208304_at	chemokine (C-C motif) receptor 3	CCR3
	216388_s_at	leukotriene B4 receptor	LTB4R
	216407_at	Vac14 homolog (S. cerevisiae)	VAC14
	231029_at	coagulation factor V (proaccelerin, labile factor	F5

227057_at	Rho GTPase activating protein 27	ARHGAP27
217808_s_at	mitogen-activated protein kinase associated protein 1	MAPKAP1
221568_s_at	lin-7 homolog C (C. elegans)	LIN7C
228263_at	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein	GRASP
217422_s_at	CD22 molecule	CD22
201137_s_at	major histocompatibility complex, class II, DP beta 1	HLA-DPB1
Glucose/Lipid/Protein metabolism		
225286_at		
223696_at		
225280_x_at	arylsulfatase D	ARSD
219707_at	copine VII	CPNE7
218292_s_at	protein kinase, AMP-activated, gamma 2 non-catalytic	PRKAG2
202499_s_at	solute carrier family 2 (facilitated glucose transporter), member 3	SLC2A3
203916_at	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 2	NDST2
222622_at	phosphoglycolate phosphatase	PGP
212246_at	multiple coagulation factor deficiency 2	MCFD2
201554_x_at	glycogenin 1	GYG1
1554385_a_at	peptidyl arginine deiminase, type II	PADI2
202826_at	serine peptidase inhibitor, Kunitz type 1	SPINT1
DNA processing/cell cycle		
225844_at	polymerase (DNA-directed), epsilon 4 (p12 subunit	POLE4
202769_at	cyclin G2	CCNG2
202414_at	excision repair cross-complementing rodent repair deficiency, complementation group 5	ERCC5
	histone cluster 1, H2ag	HIST1H2AG
	histone cluster 1, H2ah	HIST1H2AH
	histone cluster 1, H2ai	HIST1H2AI
	histone cluster 1, H2aj	HIST1H2AJ
	histone cluster 1, H2ak	HIST1H2AK
	histone cluster 1, H2al	HIST1H2AL
	histone cluster 1, H2am	HIST1H2AM
208583_x_at	histone cluster 1, H3f	HIST1H3F
Others		
234276_at	unknown	
227807_at	poly (ADP-ribose) polymerase family, member 9	PARP9
209264_s_at	tetraspanin 4	TSPAN4
	T cell receptor beta constant 1	TRBC1
	T cell receptor beta constant 2	TRBC2
	T cell receptor beta variable 19	TRBV19
	T cell receptor beta variable 7-4	TRBV7-4
	T cell receptor beta variable 7-6	TRBV7-6
	T cell receptor beta variable 7-7	TRBV7-7
217326_x_at	T cell receptor beta variable 7-8	TRBV7-
229668_at	hypothetical protein LOC90393	LOC9039
222252_x_at		
224513_s_at	ubiquilin 4	UBQLN4
229182_at	unknown	
222139_at	KIAA1466 gene	KIAA1466
240233_at	unknown	
237779_at	unknown	
212357_at	family with sequence similarity 168, member A	FAM168A
223351_at	chromosome 17 open reading frame 80	C17orf8
204787_at	V-set and immunoglobulin domain containing 4	VSIG4

226630_at	chromosome 14 open reading frame 106	C14orf106
1553153_at	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2	ATP6V0D2
212793_at	dishevelled associated activator of morphogenesis 2	DAAM2
223777_at	hypothetical LOC84771	MGC13005

Down-regulated

Transcription/RNA processing

1554015_a_at	chromodomain helicase DNA binding protein 2	CHD2
1560228_at	snail homolog 3 (Drosophila	SNAI3
208632_at		
207801_s_at	ring finger protein 10	RNF10
243496_at	RAB18, member RAS oncogene family	RAB18
205453_at	homeobox B2	HOXB2
242706_s_at	mediator complex subunit 23	MED23
224767_at	Ribosomal protein L37	RPL37
235623_at	Elongation protein 2 homolog (S. cerevisiae	ELP2
213529_at	zinc finger protein 688	ZNF688
241402_at	tRNA splicing endonuclease 54 homolog (S. cerevisiae	TSEN54
231203_at	similar to hCG1651427	LOC100129633
1560922_s_at	zinc finger protein 169	ZNF16
201533_at	catenin (cadherin-associated protein), beta 1, 88kDa	CTNNB1

Signal transduction

213013_at	mitogen-activated protein kinase 8 interacting protein 1	MAPK8IP1
235241_at	solute carrier family 38, member 9	SLC38A9
205336_at	parvalbumin	PVALB
214567_s_at	chemokine (C motif) ligand 1	XCL1, XCL2
225562_at	RAS p21 protein activator 3	RASA3
212841_s_at	PTPRF interacting protein, binding protein 2 (liprin beta 2)	PPFIBP2
	calmodulin 1 (phosphorylase kinase, delta)	CALM1
	calmodulin 2 (phosphorylase kinase, delta)	CALM2
213688_at	calmodulin 3 (phosphorylase kinase, delta)	CALM3
215179_x_at	Placental growth factor	PGF
201314_at	Serine/threonine kinase 25 (STE20) homolog, yeast	STK25
215404_x_at	fibroblast growth factor receptor	FGFR1
212070_at	G protein-coupled receptor 56	GPR56
212821_at	pleckstrin homology domain containing, family G (with RhoGef domain) member 3	PLEKHG3
230464_at	sphingosine-1-phosphate receptor 5	S1PR5
233101_at	myotubularin related protein 9	MTMR9
211546_x_at	synuclein, alpha (non A4 component of amyloid precursor)	SNCA
221772_s_at	protein phosphatase 2, regulatory subunit B, delta isoform	PPP2R2D
238886_at	transmembrane emp24-like trafficking protein 10 (yeast	TMED10
223041_at	CD99 molecule-like 2	CD99L2
242814_at	serpin peptidase inhibitor, clade B (ovalbumin), member	SERPINB9
227802_at	RUN and FYVE domain containing 3	RUFY3
220646_s_at	killer cell lectin-like receptor subfamily F, member 1	KLRF1
227259_at	CD47 molecule	CD47
225097_at	homeodomain interacting protein kinase 2	HIPK2
203235_at	thimet oligopeptidase 1	THOP1
204738_s_at	KRIT1, ankyrin repeat containing	KRIT1
210235_s_at	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 1	PPFIA1
	G protein-coupled receptor 89A	GPR89A
223531_x_at	G protein-coupled receptor 89B	GPR89B

	G protein-coupled receptor 89C	GPR89C
Glucose/Lipid/Protein metabolism		
234762_x_at	Neurolysin (metallopeptidase) M3 family	NLN
1558561_at	histocompatibility (minor) 13	HM13
202323_s_at	acyl-Coenzyme A binding domain containing 3	ACBD3
206709_x_at	glutamic-pyruvate transaminase (alanine aminotransferase	GPT
204142_at	enolase superfamily member	ENOSF1
227960_s_at	fumarylacetoacetate hydrolase domain containing 1	FAHD1
203676_at	glucosamine (N-acetyl)-6-sulfatase	GNS
228595_at	hydroxysteroid (17-beta) dehydrogenase 1	HSD17B1
218533_s_at	uridine-cytidine kinase 1-like 1	UCKL1
202735_at	emopamil binding protein (sterol isomerase	EBP
214765_s_at	N-acylethanolamine acid amidase	NAAA
202458_at	protease, serine, 23	PRSS23
Others		
	hect domain and RLD 2 pseudogene 2	HERC2P2
	hect domain and RLD 2 pseudogene 3	HERC2P3
217317_s_at	hect domain and RLD 2 pseudogene	LOC440248
214695_at	ubiquitin associated protein 2	UBAP2L
232516_x_at	unknown	
231225_at	Unknown	
236191_at	Unknown	
242558_at	Unknown	
240061_at	Pigeon homolog (Drosophila	PION
222559_s_at	regulation of nuclear pre-mRNA domain containing 1A	RPRD1A
230388_s_at	hypothetical protein LOC644246	LOC644246
233319_x_at	Unknown	
233554_at	Unknown	
229063_s_at	coiled-coil domain containing 107	CCDC107
213367_at	hypothetical LOC791120	LOC791120
1562849_at	Unknown	
240326_at	Unknown	
232775_at	Unknown	
214656_x_at	myosin IC	MYO1C
203940_s_at	vasohibin 1	VASH1
205010_at	guanine nucleotide binding protein-like 3 (nucleolar) like	GNL3L
230946_at	Formin 2	FMN2
217446_x_at	Unknown	
217891_at	chromosome 16 open reading frame 58	C16orf58
223039_at	chromosome 22 open reading frame 13	C22orf1
240188_at	Unknown	
215587_x_at	Unknown	
223175_s_at	fem-1 homolog a (C. elegans)	FEM1A
205495_s_at	Granulysin	GNLY
233757_x_at	Unknown	
215204_at	Unknown	
207840_at	CD160 molecule	CD160
204461_x_at	RAD1 homolog (S. pombe)	RAD1
243088_at	Unknown	
237588_at	Unknown	
222781_s_at	chromosome 9 open reading frame 40	C9orf4
220696_at	PRO0478 protein	PRO0478
	hypothetical LOC647081	
	succinate dehydrogenase complex, subunit D, integral	LOC647081
215652_at	membrane protein	SDHD
37943_at	zinc finger, FYVE domain containing 26	ZFYVE26
218519_at	solute carrier family 35, member A5	SLC35A5
232253_at	Hypothetical gene supported by AK128882	LOC441108

	244633_at	Unknown	
	242077_x_at	chromosome 6 open reading frame 150	C6orf15
	221824_s_at	membrane-associated ring finger (C3HC4) 8	MARCH8
	239591_at	Unknown	
	209516_at	SMYD family	SMYD5
	226928_x_at	Solute carrier family 25, member 37	SLC25A37
	1557457_at	Unknown	
	228788_at	yippee-like 1 (Drosophila)	YPEL1
	1558525_at	hypothetical protein LOC283901	LOC283901
	228947_x_at	transmembrane protein 204	TMEM204
	241373_at	Unknown	
	222692_s_at	fibronectin type III domain containing 3B	FNDC3B
	229035_s_at	kelch domain containing	KLHDC4
	203919_at	transcription elongation factor A (SII), 2	TCEA2
	1553177_at	SH2 domain containing 1B	SH2D1B
	1562505_at	Unknown	
	216586_at	Unknown	
	239137_x_at	chromosome 6 open reading frame 223	C6orf223
	221992_at	similar to nuclear pore complex interacting protein	LOC440348
	1556588_at	chromosome 15 open reading frame 37	C15orf37
	236966_at	armadillo repeat containing 8	ARMC8
	231658_x_at	Unkkown	
	233321_x_at	hypothetical protein BC001742	LOC90834