RBMXL1 is differentially expressed in the brains of patients with psychotic disorders.

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We used public and published microarray data^{1,2} to identify the most significant gene expression changes in the brains of patients with psychotic disorders. We identified RBMXL1 as differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder. In neurons of the dorsolateral prefrontal cortex, RBMXL1 was expressed at significantly lower levels in patients with psychotic disorders.

Keywords: schizophrenia, psychotic disorders, systems biology of schizophrenia, psychosis, RBMXL1.

Psychotic disorders including schizophrenia and schizoaffective disorder are characterized by the presence of psychosis, or an inability to engage in reality testing^{3,4}. Patients with psychotic disorders can experience positive as well as negative symptoms⁵. Positive symptoms include hallucinations, visual and auditory, as well as delusions, or fixed false beliefs⁵. Negative symptoms include anhedonia, or an inability to experience pleasure, as well as avolition, or loss of motivation⁵. To understand how specific regions of the brain differ most significantly in terms of gene expression in patients with psychotic disorders, we used published and public microarray data to compare global gene expression profiles of brain tissues from patients with psychotic disorders with that of non-affected, control subjects^{1,2}. We found that RBMXL1 was among the genes whose expression changed most significantly in patients with schizophrenia in the dorsolateral prefrontal cortex, the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and in the layer 3 and layer 5 pyramidal neurons of the DLPFC in patients with schizophrenia. RBMXL1 may be relevant for future study of genes pertinent to the biology of psychotic disorders.

Methods

We used microarray datasets GSE93577 (1) and GSE87610 (2) for this differential gene expression analysis in conjunction with GEO2R. GSE93577 was generated using Affymetrix Human Genome U219 Array technology with n=68 for control subjects and n=70 for patients with schizophrenia and schizoaffective disorders, using layer 3 (L3) parvalbumin neurons of the dorsolateral prefrontal cortex (DLPFC). GSE87610 was generated using Affymetrix Human

Genome U219 with n=72 for control subjects and n=65 for patients with schizophrenia, using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether RBMXL1 expression was significantly different when comparing neurons from the DLPFC from control subjects and or from patients with schizophrenia or schizophrenia and schizoaffective disorder, respectively, using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0) (455).

Results

We mined published and public microarray datasets to identify differentially expressed genes in the brains of patients with psychotic disorders.

RBMXL1 is differentially expressed in the PV+ L3 neurons dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder.

We identified RBMXL1 as among the genes whose expression changed most significantly genome-wide when comparing the transcriptional profiles of parvalbumin-positive L3 neurons of the dorsolateral pre-frontal cortex (DLPFC) from control, non-affected subjects and from patients with schizophrenia or schizoaffective disorder (Table 1). When sorting each

1 of the transcripts identified by microarray based on degree of change in expression between
2 control subjects and patients with psychotic disorders, RBMXL1 ranked 147 out of 49386 total
3 transcripts (Table 1). Differential expression of RBMXL1 in the PV+ L3 neurons of the DLPFC
4 of patients with psychotic disorders in this dataset was statistically significant (Table 1;
5 $p=9.23E-05$).

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9 RBMXL1 is differentially expressed in the L3 and L5 pyramidal neurons of the dorsolateral
10 prefrontal cortex in patients with schizophrenia.

11 We mined a second microarray dataset to determine if differential expression of
12 RBMXL1 in patients with psychotic disorders could be observed. We found here that RBMXL1
13 was among the genes most differentially expressed when comparing L3 and L5 pyramidal
14 neurons from patients with schizophrenia to that of normal subjects. When sorting each of the
15 transcripts identified by microarray based on degree of change in expression between control
16 subjects and patients with schizophrenia, RBMXL1 ranked 3240 out of 49386 total transcripts
17 (Table 1). Differential expression of RBMXL1 in the DLPFC of patients with schizophrenia was
18 statistically significant (Table 2; $p=1.39E-02$).

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22 RBMXL1 expression is significantly lower in the PV+ L3 neurons dorsolateral prefrontal
23 cortex of patients with schizophrenia and schizoaffective disorder.

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25 We obtained exact mRNA expression levels for the differentially expressed RBMXL1
26 transcript, from control subjects and from patients with schizophrenia, to directly compare
27 RBMXL1 expression levels in the PV+ L3 neurons of the DLPFC. We also performed a
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1 statistical test to determine if this difference in RBMXL1 expression was statistically significant.
2 RBMXL1 was expressed at lower levels in the PV+ L3 neurons of the DLPFC in patients with
3 schizophrenia, and this difference was statistically significant (Figure 1; $p=0.0001$). We
4 calculated a mean fold change of 0.8910 ± 0.1517 in RBMXL1 expression when comparing
5 substantia nigra from patients with schizophrenia to that of control subjects (Table 1).
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8 Thus, we found that RBMXL1 was among the most differentially expressed genes in the
9 brains of patients with psychotic disorders, both in the dorsolateral prefrontal cortex and in the
10 parvalbumin-positive L3 neurons of the dorsolateral prefrontal cortex, and that RBMXL1 was
11 expressed at significantly lower PV+ L3 neurons of the DLPFC in patients with schizophrenia.
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14 **Discussion**

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16 Retrogenes are processed copies of other genes, generated by insertion of copies of
17 messenger RNA into the genome⁶. RNA binding motif protein, X-linked-like-1 (RBMXL1) is a
18 nuclear RNA binding protein and retrogene of the RNA-binding motif protein X-linked RBMX
19 containing an RNA-binding motif (RBM)⁷. RBMX, also known as heterogenous
20 ribonucleoprotein G (hnRNP G), and found on the X chromosome is a homolog of RBMY, an
21 RBM protein found on the Y chromosome⁷. RBMX-like sequences, like RBMXL1, lack intronic
22 sequences in their genomic organization, demonstrating that they arose from retrotransposition⁷.
23 RBMX and RBMY are an example of an X/Y gene pair, wherein the Y copy acquires a testis-
24 specific function while the X copy is subject to X inactivation in females.
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1 RBMXL1 has 96% identity at the nucleotide level with RBMX and encodes a protein of 390
2 amino acids in length⁷.

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4 There is little information available about the specific function of RBMXL1, but
5 information regarding RBMXL1 can be gleaned by study of its derivative gene, RBMX. RBMX
6 is glycosylated and possesses an RBM at its amino-terminus and a carboxy-terminus rich in
7 serine, arginine and glycine⁸. hnRNP G is a reader of the N⁶-methyladenosine modification
8 through interactions with RNA via the RBM and RGG (arginine glycine glycine) motifs⁸.
9 hnRNP G can bind the carboxy-terminus of RNA polymerase II (RNAPII) once phosphorylated,
10 co-transcriptionally associating with RNAPII and modulating RNA splicing; m⁶A sites near
11 splice sites can thus affect hnRNP G binding⁸. RBMX can function as a regulator of the DNA
12 damage response, localizing to DNA lesions and promoting homologous recombination through
13 mechanisms involving BRCA2 expression dependent on poly (ADP-ribose) polymerase-1
14 (PARP-1)⁹. RBMX can also influence chromosome morphogenesis through interaction with the
15 cohesin regulator Wapl and with cohesin subunits Scc1 and Smc3; depletion of RBMX results in
16 loss of cohesin from centromeres before anaphase¹⁰. In amphibian oocytes, a central nascent
17 RNA targeting domain (NTD) and not the RBM is required for hnRNP G interaction with
18 nascent transcripts¹¹. One study implicated RBMX and RBMXL1 in maintenance of
19 “sonication-resistant” heterochromatin marked by H3K9me₃; RBMX had the second highest
20 enrichment at H3K9me₃-marked chromatin; knockdown of RBMX and RBMXL1 enhanced
21 expression of liver genes in fibroblasts, demonstrating that RBMX and RBMXL1 maintenance
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1 of sonication-resistant heterochromatin could serve as an impediment to reprogramming¹².

2 Rbm^x is expressed in the branchial arches and primordium in the developing zebrafish
3 brain and expression of forebrain and hindbrain markers *otx2* and *krox20* are decreased in
4 Rbm^x-deficient zebrafish¹³. In *Xenopus*, RBMX is also required for patterning of the anterior
5 neural plate, neural crest development and muscle segmentation¹⁴. RBMX is a candidate gene
6 for a form of moderate intellectual disability known as Shashi X-linked intellectual disability
7 accompanied by craniofacial abnormalities¹⁵. Thus, RBMX functions in alternative splicing, can
8 interact with RNA polymerase II, can mediate homologous recombination during DNA damage
9 response, has 96% identity with its retrogene RBMX and is required for development of the
10 brain in zebrafish and for the development of the anterior neural plate in *Xenopus*.
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12 We observed significant changes in gene expression of a poorly characterized gene,
13 RBXML1, in the dorsolateral prefrontal cortex of patients with psychotic disorders, in L3 PV+
14 neurons and in L3 and L5 pyramidal neurons. RBXML1 may be of relevance to the biology of
15 psychotic disorders.
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Rank	ID	p-value	t	B	FC	Gene
147	11726096_s_at	9.23E-05	-4.03	1.228245	0.8910 ± 0.1517	RBMXL1

Table 1: RBMXL1 is differentially expressed in L3 parvalbumin neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of RBMXL1 in neurons of the DLPFC when compared to DLPFC neurons from control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
3240	11758378_s_at	1.39E-02	-2.4912189	-3.12743	RBMXL1

Table 2: RBMXL1 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex in patients with schizophrenia.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of RBMXL1 in neurons of the DLPFC when compared to DLPFC neurons from control subjects, the gene and gene name are listed in this chart.

Figure 1: RBMXL1 is expressed at significantly lower levels in the L3 PV+ neurons of the DLPFC in patients with schizophrenia and schizoaffective disorder.

The mRNA expression of RBMXL1 in L3 PV+ DLPFC neurons from control subjects (left) and in the L3 PV+ DLPFC neurons from patients with schizophrenia or schizoaffective disorder (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in RBMXL1 expression between unaffected L3 PV+ neurons of the DLPFC and L3 PV+ neurons of the DLPFC from patients with psychotic disorders, a p-value, listed above.