RNase L 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 the gene encoding the endoribonuclease RNase L as differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder. In neurons of the dorsolateral prefrontal cortex, RNase L was expressed at significantly lower levels in patients with psychotic disorders.

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

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 RNASEL, the gene encoding the ribonuclease RNase L 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. RNase L 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 RNASEL 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.

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

. We identified the endoribonuclease RNase L (encoded by RNASEL) as among the genes whose expression changed most significantly genome-wide when comparing the transcriptional profiles of parvalbumin-positive (PV+) L3 neurons of the dorsolateral pre-frontal cortex (DLPFC) from control subjects and from patients with schizophrenia or schizoaffective disorder (Table 1). When sorting each of the transcripts identified by microarray based on

significance of change in expression between control subjects and patients with psychotic disorders, RNASEL ranked 243 out of 49386 total transcripts (Table 1). Differential expression of RNASEL in the PV+ L3 neurons of the DLPFC in patients with psychotic disorders here was statistically significant (Table 1; $p=1.72E-04$).

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

We mined a second microarray dataset to determine if differential expression of RNASEL in patients with psychotic disorders could be observed. We found here that RNASEL was among the genes most differentially expressed when comparing L3 and L5 pyramidal neurons from patients with schizophrenia to that of normal subjects. When sorting each of the transcripts identified by microarray based on degree of change in expression between control subjects and patients with schizophrenia, RNASEL ranked 5253 out of 49386 total transcripts (Table 1). Differential expression of RNASEL in the DLPFC of patients with schizophrenia was statistically significant (Table 2; $p=3.17E-02$).

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

We obtained exact mRNA expression levels for the differentially expressed RNASEL transcript, from control subjects and from patients with schizophrenia, to directly compare RNASEL expression levels in the PV+ L3 neurons of the DLPFC. We also performed a statistical test to determine if this difference in RNASEL expression was statistically significant.

1 RNASEL was expressed at lower levels in the PV+ L3 neurons of the DLPFC in patients with
2 schizophrenia, and this difference was statistically significant (Figure 1; $p=0.0002$). We
3 calculated a mean fold change of 0.8923 ± 0.1066 in RNASEL expression when comparing
4 substantia nigra from patients with schizophrenia to that of control subjects (Table 1).
5

6 Thus, we found that RNASEL was among the most differentially expressed genes in the
7 brains of patients with psychotic disorders, both in the dorsolateral prefrontal cortex and in the
8 parvalbumin-positive L3 neurons of the dorsolateral prefrontal cortex, and that RNASEL was
9 expressed at significantly lower PV+ L3 neurons of the DLPFC in patients with schizophrenia.
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12 **Discussion**

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14 We observed significant changes in gene expression of RNASEL in the dorsolateral
15 prefrontal cortex of patients with psychotic disorders, in L3 PV+ neurons and in L3 and L5
16 pyramidal neurons.
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18 RNase L functions as a 2',5'-oligoadenylate endoribonuclease that cleaves single-
19 stranded RNA in the interferon-induced 2-5A pathway⁶. Production of short, 2',5'-linked
20 oligoadenylates (2-5A) by 2',5'-oligoadenylate synthetase (OAS) using ATP following 2',5'-
21 OAS activation by double stranded RNA activates the endoribonuclease function of RNase L⁶.
22 Mice deficient in RNase L display deficits in interferon signaling and in cell death (apoptosis),
23 consistent with the described role of RNase L as an antiviral endoribonuclease associated with
24 the interferon signaling response^{7,8}; in 3T3/pLZ cells, high expression of RNase L could inhibit
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1 replication of encephalomyocarditis virus, vesicular stomatitis virus, human parainfluenza virus-3
2 and vaccinia virus⁸. 2-5A activation of RNase L can also result in the production of cleaved self
3 RNA fragments that trigger synthesis of interferon beta through RIG-1, MDA5 and MAVS
4 (IPS-1)⁹. Induction of cell death through 2-5A activation of RNase L involves release of
5 cytochrome c from the mitochondria with ensuing caspase-3 activation and subsequent
6 apoptosis¹⁰.

9 RNase L can regulate protein translation through interaction with the translation
10 termination factor eRF3/GSPT1¹¹. This interaction results in more efficient translation read
11 through at premature stop codons as well as enhanced +1 frameshift site efficiency, suggesting
12 RNase L functions in regulating translation termination¹¹.

15 Another function of RNase L is in triggering activation of innate immune signaling
16 through the NLRP3 inflammasome during viral infections¹². RNA cleavage products generated
17 by RNase L possess 2',3'-cyclic phosphorylated termini and enhance association of NLRP3
18 complex with DHX33 and MAVS¹².

20 RNase L can modulate severity of brain pathology in viral infection. In mice deficient in
21 RNase L, while RNase L deficiency did not significantly alter infectivity of herpes simplex 2
22 virus (HSV-2) *in vivo*, neurologic disease associated with HSV-2 infection was less severe,
23 demonstrating that RNase L could influence disease outcome in the brain associated with the
24 inflammatory response to viral infection *in vivo*¹³.

27 Infection of RNase L deficient mice with a demyelinating variant of mouse hepatitis virus
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1 resulted in significantly shorter survival; RNase L deficiency did not change viral control or
2 interferon production but mice deficient in RNase L featured early onset of and more severe
3 demyelination as well as axonal damage in the brain stem and spinal cord¹⁴.
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5 Mice deficient in both protein kinase R (PKR) and RNase L have a 90% mortality rate
6 after infection with the neurotropic flavivirus West Nile Virus (WNV) as compared to 30%
7 mortality in wild-type mice¹⁵. Neuronal tissues from the central nervous system of PKR-/-
8 RNase L -/- mice displayed higher viral burden than wild-type mice. However, mice solely
9 deficient for RNase L were less susceptible than mice deficient in both PKR and RNase L to
10 subcutaneous WNV infection, indicating that at least part of viral control was attributable to
11 functions of PKR.
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15 We found significant differential expression of RNase L in neurons from patients with
16 psychosis. If validated, neurons produced by *in vitro* differentiation of patient fibroblast-derived
17 induced pluripotent stem cells from patients with schizophrenia or schizoaffective disorder can
18 be utilized to understand *in vitro* what effect changes in RNase L expression have on response to
19 viral infections or inflammatory triggers without the need for death to transpire before studying
20 patient tissues.
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Rank	ID	p-value	t	B	FC	Gene
243	11734359_a_at	1.72E-04	-3.86	0.681279	0.8923 ± 0.1066	RNASEL

Table 1: RNASEL 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 RNASEL 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
5253	11734358_a_at	3.17E-02	2.1704383	-3.80596	RNASEL

Table 2: RNASEL 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 RNASEL 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: RNASEL 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 RNASEL 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 RNASEL 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.