1 The serine/threonine kinase 39, STK39, is differentially expressed in the brains of patients with
2 schizophrenia.

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6 Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined
7 published and public microarray datasets^{2,3} to discover differentially expressed genes in
8 schizophrenia and schizoaffective disorder. We found significant differential expression of the
9 serine/threonine kinase 39, STK39, in neurons of the dorsolateral pre-frontal cortex from patients
10 with schizophrenia and schizoaffective disorder.
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27 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
28 biology of schizophrenia, STK39.

Psychiatric diseases schizophrenia and schizoaffective disorder have in common the existence of psychotic symptoms^{1,4,5}, like visual and auditory hallucinations, that can plague affected patients. Understanding the transcriptional profiles of cells and tissues from the brain of patients diagnosed with schizophrenia or schizoaffective disorder has the potential to provide insights into molecular mechanisms underlying disease initiation, progression and associated symptoms like psychosis. We mined published and public microarray data from layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder and from the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex of patients with schizoaffective disorder to discover genes whose expression was most significantly different in the brains of patients with psychosis at the systems-level. STK39 was among the most differentially expressed genes in patients with psychotic disorders in two cell types of the brain.

Methods

We used microarray datasets GSE93577² and GSE87610³ for this differential gene expression analysis in conjunction with GEO2R. GSE93577 was generated using Affymetrix Human Genome U219 Array technology from layer 3 (L3) parvalbumin (PV) positive neurons of the dorsolateral prefrontal cortex, and we used $n=34$ control subjects each with paired replicates ($n=68$ total samples) and $n=35$ patients with schizophrenia or schizoaffective disorder with paired replicates ($n=70$ total samples) from this dataset. These patient diagnoses in this dataset included schizoaffective disorder, paranoid schizophrenia, undifferentiated schizophrenia,

1 chronic paranoid schizophrenia, chronic disorganized schizophrenia, chronic undifferentiated
2 schizophrenia, and disorganized schizophrenia. GSE87610 was generated using Affymetrix
3 Human Genome U219 with $n=72$ for control subjects and $n=65$ for patients with schizophrenia,
4 using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex.
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6 The Benjamini and Hochberg method of p-value adjustment was used for ranking of
7 differential expression but raw p-values were used for assessment of statistical significance of
8 global differential expression. Log-transformation of data was auto-detected, and the NCBI
9 generated category of platform annotation was used. A statistical test was performed to evaluate
10 whether STK39 expression was significantly different when comparing neurons of the DLPFC
11 from control subjects and from patients with schizophrenia and schizoaffective disorder using a
12 two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses
13 (Version 8.4.0)(455).
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19 Results

20 We mined published and public microarray data^{2,3} to identify in an unbiased fashion and
21 at the global level genes most differentially expressed in the brains of patients with schizophrenia
22 and schizoaffective disorder.
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25 STK39 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral
26 pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.
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1 We observed differential expression of the serine/threonine kinase 39, encoded by the
2 STK39 gene, in the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in
3 patients with schizophrenia and schizoaffective disorder². When sorting each of the transcripts
4 whose expression was measured by microarray based on significance of change in expression
5 between diseased and control patients, in L3 PV+ DLPFC neurons from patients with
6 schizophrenia, STK39 ranked 15 out of 49386 total transcripts. Differential expression of
7 STK39 was statistically significant (Table 1; $p=3.34E-06$).
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12 STK39 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal
13 cortex in patients with schizophrenia.
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15 We also observed differential expression of STK39 in the layer 3 and layer 5 (L5)
16 pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the
17 transcripts whose expression was measured by microarray based on significance of change in
18 expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC
19 from patients with schizophrenia, STK39 ranked 4785 out of 49386 total transcripts (Table 2),
20 equating to 90.3% differential expression. Differential expression of STK39 in L3 and L5
21 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant
22 (Table 2; $p=2.72E-02$).
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26 STK39 expression is significantly lower in layer 3 parvalbumin-positive neurons of the
27 dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.
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We obtained exact mRNA expression levels for the differentially expressed STK39 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare STK39 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in STK39 expression was statistically significant. STK39 was expressed at lower levels in the L3 PV+ DLPFC neurons in patients with schizophrenia and schizoaffective disorder, and this difference was statistically significant (Figure 1; $p < 0.0001$). We calculated a mean fold change of 0.9 ± 0.1 in STK39 expression when comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder to that of control subjects (Table 1).

Thus, we found significantly differential expression of STK39 in two types of neuronal cell types in patients with schizophrenia; STK39 expression was significantly lower in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

STK39, also known as STE20/SPS1-related proline alanine-rich kinase, or SPAK, was cloned from a pancreatic beta cell line with expression in the brain, pancreas, heart, lung skeletal muscle and kidney⁶, and also identified as an STE-related protein kinase with expression in the neurons, epithelial cells of the choroid plexus, collecting duct and distal tubule of the kidneys, salivary gland duct and parietal cells of the stomach, and containing an alanine and proline rich

1 domain at its amino-terminus. STK39 spans 547 amino acids with a molecular weight of 59.6
2 kilodaltons, contains an amino-terminal PAPA box, a kinase domain, and an acid box within
3 which a caspase cleavage site lies, between its catalytic site and the carboxy-terminus⁶. STK39
4 is phylogenetically related to the MST kinase family and is a member of the STE20 kinase SPS1
5 subfamily⁶. STK39 immunoprecipitated from COS7 cells can activate the p38 MAP kinase⁶.
6 While a STK39 GFP fusion localizes to the cytoplasm of COS7 cells, a carboxy-terminally
7 truncated STK39 GFP fusion that replicates the product of caspase cleavage localizes to the
8 nucleus, suggesting caspase cleavage of STK39 induces its nuclear localization⁶. A yeast two-
9 hybrid screen utilizing a Jurkat T-cell library identified STK39 as a protein kinase C theta
10 (PKC θ)-interacting protein, and endogenous STK39 associates with PKC θ in Jurkat cells⁸.
11 STK39 can be phosphorylated by PKC θ ⁸. In Jurkat-TAg cells transfected with CD28, anti-CD3
12 and anti-CD28 antibody stimulation resulted in STK39 activation, suggesting T-cell stimulation
13 through CD3 and CD28 could activate STK39⁸. STK39 activation through CD3/CD8 co-
14 stimulation required its PKC θ -interacting domain and serine 311, and consistent with this,
15 activation of STK was defective in PKC θ -deficient T-cells; the downstream result of STK39
16 activation through PKC θ in T-cells was modulation of AP-1 signaling and not NF-kB signaling⁸.
17 Another name for STK39 or SPAK is the proline- and alanine-rich Ste20-related kinase, or
18 PASK⁹. In the rat brain, STK39 can be found in association with complexes containing actin and
19 tubulin, and a GST-STK39 fusion protein can interact with F-actin; nearly half of STK in a rat
20 brain fraction can be found in a Triton X-100 insoluble fraction⁹. Under conditions of cellular
21 stress, including heat shock and exposure to hydrogen peroxide, cytosolic STK39 becomes
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1 associated with the cytoskeleton⁹. Another interaction partner of STK39 is the Na⁺ K⁺ 2Cl⁻ co-
2 transporter (NKCC1), but co-transporter function was reported to be not affected by inability to
3 bind STK39¹⁰. While STK39 and NKCC1 binding is unaffected by cellular stress, the quantity
4 of p38 MAP kinase is reduced¹⁰. The WNK1 and WNK4 kinases can phosphorylate and activate
5 SPAK¹¹.

8 Mice with one copy of STK39 are hypotensive, and STK39-null mice manifest
9 phenotypes that bear similarities to patients with Gitelman syndrome, with hypokalemia,
10 hypomagnesia, and hypocalcuria¹². The nociceptive threshold of STK39-deficient mice is higher
11 than in wild-type mice as evidenced by hot plate and tail flick assays; in addition, locomotive
12 function is defective in STK-deficient mice as evidenced by decreased performance on a rotarod
13 assay and decreased activity in open field assays¹³. Importantly, STK39-deficient mice display
14 increased anxiety-like behaviors, with increased area in the dark portion of a light/dark box¹³.
15 STK39 is expressed in the spinal cord and dorsal root ganglion (DRG) neurons; in this study,
16 however, the activity of NKCC1 was reduced by approximately one half in STK39-deficient
17 DRG neurons¹⁴. Thus, STK39 is expressed in the brain, can activate p38 MAP kinase, may be
18 subject to caspase cleavage, can associate with F-actin, has function associated with the NKCC1
19 in neuronal cells, and STK39-deficient mice manifest anxiety-like behaviors.

24 We found differential expression of STK39 in neurons from patients with schizophrenia
25 in two published or public microarray datasets. Differential expression of STK39 should be
26 validated in separate and larger schizophrenia patient datasets. STK39 may be of relevance to
27 the biology of schizophrenia.
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Rank	ID	p-value	t	B	FC	Gene
15	11717956_s_at	3.34E-06	-4.85	4.158939	0.9 ± 0.1	STK39

Table 1: STK39 is differentially expressed in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

The rank of differential expression, the probe 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 STK39 in L3 PV+ DLPFC neurons of patients with schizophrenia and schizoaffective disorder when compared to deep pyramidal neurons from control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
4785	11755400_s_at	2.72E-02	-2.2325658	-3.68127	STK39

Table 2: STK39 is differentially expressed in the layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia.

The rank of differential expression, the probe 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 gene and gene name are listed in this chart.

Figure 1: STK39 is expressed at significantly lower levels in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia and schizoaffective disorder.

The mRNA expression of STK39 in L3 PV+ neurons of the DLPFC from control subjects (left) and from patients with schizophrenia and schizoaffective disorder (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in STK39 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.