1 Wdr47 is differentially expressed in the brains of patients with schizophrenia.

2 Shahan Mamoor, MS¹

3 ¹shahanmamoor@gmail.com

4 East Islip, NY USA

5 Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined
6 published and public microarray datasets^{2,3} to discover differentially expressed genes in
7 schizophrenia and schizoaffective disorder. We found significant differential expression of
8 Wdr47 in neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and
schizoaffective disorder.

26 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
27 biology of schizophrenia, Wdr47.

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. Wdr47 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, chronic paranoid schizophrenia, chronic disorganized schizophrenia, chronic undifferentiated

1 schizophrenia, and disorganized schizophrenia. GSE87610 was generated using Affymetrix
2 Human Genome U219 with $n=72$ for control subjects and $n=65$ for patients with schizophrenia,
3 using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex.
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5 The Benjamini and Hochberg method of p-value adjustment was used for ranking of
6 differential expression but raw p-values were used for assessment of statistical significance of
7 global differential expression. Log-transformation of data was auto-detected, and the NCBI
8 generated category of platform annotation was used. A statistical test was performed to evaluate
9 whether Wdr47 expression was significantly different when comparing neurons from the DLPFC
10 from control subjects and or from patients with schizophrenia or schizophrenia and
11 schizoaffective disorder, respectively, using a two-tailed, unpaired t-test with Welch's correction.
12 We used PRISM for all statistical analyses (Version 8.4.0)(455).
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17 **Results**

18 We mined published and public microarray data^{2,3} to identify in an unbiased fashion and
19 at the global level genes most differentially expressed in the brains of patients with schizophrenia
20 and schizoaffective disorder.
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24 Wdr47 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral pre-
25 frontal cortex in patients with schizophrenia and schizoaffective disorder.
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We observed differential expression of Wdr47 in the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in patients with schizophrenia and schizoaffective disorder². When sorting each of the transcripts whose expression was measured by microarray based on significance of change in expression between diseased and control patients, in L3 PV+ DLPFC neurons from patients with schizophrenia. Wdr47 ranked 36 out of 49386 total transcripts. Differential expression of Wdr47 was statistically significant (Table 1; $p=1.26E-05$).

Wdr47 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

We also observed differential expression of Wdr47 in the layer 3 and layer 5 (L5) pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the transcripts whose expression was measured by microarray based on significance of change in expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC from patients with schizophrenia, Wdr47 ranked 2784 out of 49386 total transcripts (Table 3). Differential expression of Wdr47 in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=1.09E-02$).

Wdr47 expression is significantly lower in layer 3 parvalbumin-positive neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

We obtained exact mRNA expression levels for the differentially expressed Wdr47 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare Wdr47 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in Wdr47 expression was statistically significant. Wdr47 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 0.8864 ± 0.1412 in Wdr47 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 Wdr47 in two types of neuronal cell types in patients with schizophrenia; Wdr47 expression was significantly lower in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

WD repeat proteins are a family of evolutionarily conserved proteins in eukaryotes that possess WD domains, repeats of tryptophan and aspartic acid which form β -propellers, serving as platforms for protein interaction and complex assembly^{6,7}. WD repeat proteins function in diverse roles including signal transduction, gene expression and vesicular transport^{6,7}. Wdr47 has described functions in the brain^{8,9} and bears structural homology to LIS1, mutation of which

1 results in lissencephaly¹⁰; LIS1 possesses WD repeats as well as an amino-terminal domain
2 known as a LisH domain¹⁰. Similarly, Wdr47 in addition to WD repeats possesses a LisH
3 domain as well as domain termed the C-terminal to LisH domain (CTLH)⁸.

5 Wdr47 is critical for establishing polarity of neurons through control of microtubule
6 dynamics⁹. In unpolarized neurons, Wdr47 functions through Camsap minus-end-binding
7 proteins to regulate microtubule organization; specifically, Wdr47 interacts with microtubules
8 through Camsap3⁹. Wdr47 can also interact with cervical ganglion-10 (SCG10), a protein that
9 has a microtubule destabilizing effect⁸. Enforced expression of Wdr47 in wild-type neurons
10 results in impairment of axon specification⁹. Mice deficient in Wdr47 die at birth due to
11 suffocation resulting from defects of the central nervous system⁹. These defects include failure
12 of axonal tract genesis and impaired radial migration⁹. Wdr47^{-/-} mice manifest impairment of
13 both migration of neurons in the neocortex as well as in the transition to bipolar and multipolar
14 neurons in the intermediate zone⁹. Wdr47^{-/-} mice also display agenesis of the corpus callosum,
15 or failure to develop the bundles of fibers that connect the left and right hemispheres of the brain;
16 severe microcephaly, thinning of cortices and impairment of sensory motor gating are other
17 features of the brain of Wdr47-deficient mice⁸. Expression of Wdr47 increases from embryonic
18 day 12.5 (E12.5) until postnatal day 2, with a peak at E18.5. Wdr47 may also be involved in
19 p62-mediated autophagy; the expression of p62 and LC-II in Wdr47 knockout mice are
20 decreased as compared to wild-type, and primary cortical neurons display abnormal
21 autophagosome formation on transmission electron microscopy⁸. Importantly, Wdr47 deficiency
22 in mice results in growth cone collapse in the cortex and hippocampus, and at E14.5 and E16.5

1 the number of axonal processes are decreased⁸. Thus, Wdr47 is an important regulator of
2 neuronal architecture in the mammalian brain.

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4 We found differential expression of Wdr47 in neurons from patients with schizophrenia
5 in two published or public microarray datasets. Differential expression of Wdr47 should be
6 validated in separate and larger schizophrenia patient datasets. Wdr47 may be of relevance to
7 the biology of schizophrenia.
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Rank	ID	p-value	t	B	FC	Gene
36	11721259_a_at	1.26E-05	-4.53	2.984609	0.8864 ± 0.1412	WDR47

Table 1: Wdr47 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 Wdr47 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
2784	11721259_a_at	1.09E-02	-2.5824263	-2.91898	WDR47

Table 2: Wdr47 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: Wdr47 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 Wdr47 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 Wdr47 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.