1 The Eps15 homology (EH) domain-containing protein 3, EHD3 is differentially expressed in the
2 brains of patients with 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 gene encoding the Eps15 homology (EH) domain-containing protein, EHD3 in neurons of the
10 dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder.

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27 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
28 biology of schizophrenia, EHD3.

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. EHD3 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 EHD3 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 EHD3 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral pre-
26 frontal cortex in patients with schizophrenia and schizoaffective disorder.
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We observed differential expression of the gene encoding the Eps15 homology (EH) domain-containing protein, EHD3, 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, EHD3 ranked 19 out of 49386 total transcripts, equating to 99.96% differential expression. Differential expression of EHD3 was statistically significant (Table 1; $p=5.48E-06$).

EHD3 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 EHD3 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, EHD3 ranked 5732 out of 49386 total transcripts (Table 2), equating to 88.4% differential expression. Differential expression of EHD3 in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=3.65E-02$).

1 EHD3 expression is significantly lower in layer 3 parvalbumin-positive neurons of the
2 dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

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4 We obtained exact mRNA expression levels for the differentially expressed EHD3
5 transcript, from control subjects and from patients with schizophrenia and schizoaffective
6 disorder, to directly compare EHD3 expression levels in L3 PV+ DLPFC neurons. We also
7 performed a statistical test to determine if this difference in EHD3 expression was statistically
8 significant. EHD3 was expressed at lower levels in the L3 PV+ DLPFC neurons in patients with
9 schizophrenia and schizoaffective disorder, and this difference was statistically significant
10 (Figure 1; $p < 0.0001$). We calculated a mean fold change of 0.9 ± 0.1 in EHD3 expression when
11 comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective
12 disorder to that of control subjects (Table 1).
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16 Thus, we found significant differential expression of EHD3 in two types of neuronal cell
17 types in patients with schizophrenia; EHD3 expression was significantly lower in L3 PV+
18 neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective
19 disorder.
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22 **Discussion**

23 EHD3 has important functions in cell biology. Two exons, identified based on exon
24 trapping using a bacterial artificial chromosome construct containing a candidate glioma tumor
25 suppressor, were utilized to screen a human fetal brain complementary DNA (cDNA) library
26 which lead to the further identification of three genes termed EHD2, EHD3 and EHD4 based on
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1 their homology to human EHD1⁵. Expression of EHD3 was relatively highest in the brain and in
2 the heart, and structurally, EHD3 contains an amino-terminal nucleotide binding sequence, a
3 nuclear localization signal and a carboxy-terminal Eps15 homology (EH) protein-binding
4 domain and EF-hand motif⁵; EHD3 can bind ATP⁶. EHD3 is likely a membrane tubulating
5 protein⁷. One interaction partner of EHD3 is Rab11-FIP2, involving NPF motif interactions in
6 Rab11-FIP2; Rab11-FIP2 can be recruitment to membranes containing EHD3, and depletion of
7 EHD3 results in the inability of endocytosed transferrin to enter the endocytic recycling
8 compartment (ERC), failure of Rab11-FIP2 to localize to the ERC in perinuclear regions, and
9 altered sub-cellular localization of Rab11⁶. EHD3 can also control cellular localization of
10 sorting nexin 1 (SNX1); depletion of SNX1 results in alteration of Golgi apparatus morphology
11 and mis-localization of SNX1 to early endosomes and decreased recruitment of AP-1 γ -adaptin
12 to the Golgi apparatus⁸. In addition, cathepsin D remains localized to the Golgi apparatus while
13 normally being trafficked to lysosomes, together indicating that EHD3 could influence transport
14 of cargoes from endosomes to the Golgi apparatus as well as morphology of the Golgi
15 apparatus⁸. Another cellular function of EHD3 is in assembly of the cilium in conjunction with
16 EHD1, Rab11 and Rab8. EHD1 and EHD3 can localize to the membrane of the ciliary pocket,
17 and membrane tubulation involving EHD1 and EHD3 is critical for generation of ciliary vesicles
18 from distal appendage vesicles (DAVs); this also involves SNAP29⁹.

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26 EHD3 has been described as a tumor suppressor in the brain¹⁰, where EHD3 can enhance
27 ubiquitin-mediated degradation of the epidermal growth factor receptor (EGFR) following EGF
28 stimulation, impair activation of EGFR as measured by tyrosine phosphorylation following EGF

1 stimulation, and regulate trafficking of EGFR, potentially re-directing EGFR away from the ERC
2 and towards lysosomal compartments¹¹. One report suggested that EHD3 was important for
3 stabilization of tubular recycling endosomes (TREs)¹².

5 Descriptions of functions for EHD3 at the level of the organism are mixed; EHD3-
6 deficient mice are born in normal Mendelian ratio, with no obvious phenotypes¹³. In a study of
7 1062 Chinese Han patients with major depressive disorder (MDD), 16 genes previously
8 identified by genome-wide association study as conferring risk or protection from MDD were
9 probed for correlation in this patient population; EHD3 was 1 of 2 genes out of 16 that was found
10 to be correlated with MDD in this cohort¹⁴. In a separate study stratifying patients based on
11 gender, 2 single nucleotide polymorphisms (SNPs) in the EHD3 gene were significantly
12 associated with MDD in females and another EHD3 SNP was significantly associated with
13 anxious mood in females¹⁵. Thus, EHD3 has important functions in membrane tubulation, in
14 trafficking of cargoes in the endocytic pathway, can interact with Rab11-FIP2, and despite no
15 obvious phenotype in EHD3-deficient mice, genetic variation in EHD3 has been significantly
16 associated with major depression and anxious mood in humans.

21 We found that EHD3 was among the genes most differentially expressed, transcriptome-
22 wide, in two neuronal cell types of the dorsolateral prefrontal cortex in patients with
23 schizophrenia: the layer 3 PV+ neurons and the layer 3 and layer 5 pyramidal neurons.
24 Differential expression of EHD3 should be validated in separate and larger schizophrenia patient
25 datasets. EHD3 may be of relevance to the cell biology of the brain in schizophrenia and
26 associated psychoses: visual and auditory hallucinations.

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Rank	ID	p-value	t	B	FC	Gene
19	11746061_a_at	5.48E-06	-4.73	3.720519	0.9 ± 0.1	EHD3

Table 1: EHD3 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 EHD3 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
5732	11746061_a_at	3.65E-02	-2.112367	-3.91955	EHD3

Table 2: EHD3 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: EHD3 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 EHD3 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 EHD3 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.