1 A pseudogene of Brd7, Brd7p3 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
9 pseudogene 3 of the bromodomain containing 7 molecule, Brd7p3, in neurons of the 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, Brd7p3.

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. The Brd7p3 molecule 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

1 included schizoaffective disorder, paranoid schizophrenia, undifferentiated schizophrenia,
2 chronic paranoid schizophrenia, chronic disorganized schizophrenia, chronic undifferentiated
3 schizophrenia, and disorganized schizophrenia. GSE87610 was generated using Affymetrix
4 Human Genome U219 with $n=72$ for control subjects and $n=65$ for patients with schizophrenia,
5 using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex.
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8 The Benjamini and Hochberg method of p-value adjustment was used for ranking of
9 differential expression but raw p-values were used for assessment of statistical significance of
10 global differential expression. Log-transformation of data was auto-detected, and the NCBI
11 generated category of platform annotation was used. A statistical test was performed to evaluate
12 whether Brd7p3 expression was significantly different when comparing neurons of the DLPFC
13 from control subjects and from patients with schizophrenia and schizoaffective disorder using a
14 two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses
15 (Version 8.4.0)(455).
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20 **Results**

21 We mined published and public microarray data^{2,3} to identify in an unbiased fashion and
22 at the global level genes most differentially expressed in the brains of patients with schizophrenia
23 and schizoaffective disorder.
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27 Brd7p3 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral
28 pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

We observed differential expression of pseudogene 3 of the bromodomain containing molecule, Brd7p3, 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, Brd7p3 ranked 2 out of 49386 total transcripts. Differential expression of Brd7p3 was statistically significant (Table 1; $p=3.6E-08$).

Brd7p3 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 Brd7p3 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, Brd7p3 ranked 2948 out of 49386 total transcripts (Table 3). Differential expression of Brd7p3 in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=1.2E-02$).

Brd7p3 expression is significantly higher 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 Brd7p3 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare Brd7p3 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in Brd7p3 expression was statistically significant. Brd7p3 was expressed at higher 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 1.1126 ± 0.1150 in Brd7p3 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 Brd7p3 in two types of neuronal cell types in patients with schizophrenia; Brd7p3 expression was significantly higher in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

Brd7 is a bromodomain-containing member of Polybromo-associated BRG-1 associated factor (PBAF) SWI/SNF chromatin remodeling complexes⁶. Bromodomains have the ability to bind acetylated lysine residues on histones, and thus Brd7 is a reader of the acetyl-lysine epigenetic mark⁷. Brd7 can interact with BRCA1, and depletion of Brd7 can impair BRCA1 recruitment to the ESR1 promoter in MCF7 cells⁸. Brd7 can function as a negative regulator of phosphoinositide-3-kinase (PI3K) signaling through direct interactions at its carboxy-terminus

1 with the inter-SH2 domain of p85 α , inducing nuclear localization p85 α and thus impairing p85 α
2
3 binding to p110 β .

4 Brd7 also has tumor suppressor functions and was identified in a genetic screen for genes
5 required for oncogene-induced senescence by p53; its inhibition permits cells to bypass
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7 senescence and transform even in the presence of wild-type p53¹⁰.

8 We could not identify literature describing a role or function for Brd7p3 in the brain, but
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10 Brd7 expression is documented throughout the brain, including the hippocampus, midbrain,
11 cerebellum, medial prefrontal cortex, and caudate putamen¹¹. In Brd7-deficient mice, neuronal
12 spine density and dendritic branching are decreased in the medial prefrontal cortex, and cognitive
13 function is impaired. The expression of proteins associated with synaptic plasticity is decreased
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15 in the medial prefrontal cortex of Brd7-deficient mice, indicating Brd7 can regulate synaptic
16 plasticity¹². rs11756438, a single nucleotide polymorphism (SNP) significantly associated with
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18 risk for development of attention deficit hyperactive disorder and bipolar disorder was found to
19 be in linkage disequilibrium with a SNP at the Brd7p3 locus¹³.

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21 We found differential expression of a poorly described non-coding RNA, pseudogene 3
22 of Brd7 (Brd7p3) in neurons from patients with schizophrenia in two published or public
23 microarray datasets. Differential expression of Brd7p3 should be validated in separate and larger
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25 schizophrenia patient datasets. Brd7p3 may be of relevance to the biology of schizophrenia.
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References

1. Andreasen, N.C., Nopoulos, P., Schultz, S., Miller, D., Gupta, S., Swayze, V. and Flaum, M., 1994. Positive and negative symptoms of schizophrenia: past, present, and future. *Acta Psychiatrica Scandinavica*, 90, pp.51-59.
2. GSE93577. University of Pittsburgh, Department of Psychiatry and Neuroscience. Pittsburgh, PA. Enwright JF, Huo Z, Arion D, Corradi JP, Tseng G, Lewis DA. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE93577>.
3. Arion, D., Huo, Z., Enwright, J.F., Corradi, J.P., Tseng, G. and Lewis, D.A., 2017. Transcriptome alterations in prefrontal pyramidal cells distinguish schizophrenia from bipolar and major depressive disorders. *Biological psychiatry*, 82(8), pp.594-600.
4. Cannon, T.D., 2015. How schizophrenia develops: cognitive and brain mechanisms underlying onset of psychosis. *Trends in cognitive sciences*, 19(12), pp.744-756.
5. Arciniegas, D.B., 2015. Psychosis. *Continuum: Lifelong Learning in Neurology*, 21(3 Behavioral Neurology and Neuropsychiatry), p.715.
6. Kaeser, M.D., Aslanian, A., Dong, M.Q., Yates, J.R. and Emerson, B.M., 2008. BRD7, a novel PBAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells. *Journal of Biological Chemistry*, 283(47), pp.32254-32263.
7. Zeng, L. and Zhou, M.M., 2002. Bromodomain: an acetyl-lysine binding domain. *FEBS letters*, 513(1), pp.124-128.
8. Harte, M.T., O'Brien, G.J., Ryan, N.M., Gorski, J.J., Savage, K.I., Crawford, N.T., Mullan, P.B. and Harkin, D.P., 2010. BRD7, a subunit of SWI/SNF complexes, binds directly to BRCA1 and regulates BRCA1-dependent transcription. *Cancer research*, 70(6), pp. 2538-2547.
9. Chiu, Y.H., Lee, J.Y. and Cantley, L.C., 2014. BRD7, a tumor suppressor, interacts with p85 α and regulates PI3K activity. *Molecular cell*, 54(1), pp.193-202.
10. Drost, J., Mantovani, F., Tocco, F., Elkon, R., Comel, A., Holstege, H., Kerkhoven, R., Jonkers, J., Voorhoeve, P.M., Agami, R. and Del Sal, G., 2010. BRD7 is a candidate tumour suppressor gene required for p53 function. *Nature cell biology*, 12(4), pp.380-389.

- 1 11. Cong, P., Jie, Z., Ying, L.H., Ming, Z., Li, W.L., Hong, Z.Q., Xin, Y.Y., Wei, X., Rong, S.S.,
2 Ling, L.X. and Yuan, L.G., 2006. The transcriptional regulation role of BRD7 by binding to
3 acetylated histone through bromodomain. *Journal of cellular biochemistry*, 97(4), pp.882-892.
- 4 12. Xu, Y., Cao, W., Zhou, M., Li, C., Luo, Y., Wang, H., Zhao, R., Jiang, S., Yang, J., Liu, Y. and
5 Wang, X., 2015. Inactivation of BRD7 results in impaired cognitive behavior and reduced
6 synaptic plasticity of the medial prefrontal cortex. *Behavioural Brain Research*, 286, pp.1-10.
- 7 13. van Hulzen, K.J., Scholz, C.J., Franke, B., Ripke, S., Klein, M., McQuillin, A., Sonuga-
8 Barke, E.J., Kelsoe, J.R., Landén, M., Andreassen, O.A. and Lesch, K.P., 2017. Genetic
9 overlap between attention-deficit/hyperactivity disorder and bipolar disorder: evidence from
10 genome-wide association study meta-analysis. *Biological psychiatry*, 82(9), pp.634-641.

Rank	ID	p-value	t	B	FC	Gene
2	11763937_at	3.6E-08	5.84	8.192295	1.1126 ± 0.1150	BRD7P3

Table 1: Brd7p3 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 Brd7p3 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
2948	11755454_s_at	1.2E-02	2.5465628	-3.00175	BRD7P3

Table 2: Brd7p3 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: Brd7p3 is expressed at significantly higher levels in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia and schizoaffective disorder.

The mRNA expression of Brd7p3 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 Brd7p3 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.