1 The type 2 deiodinase (D2), Dio2, is differentially expressed in the brains of patients with
2 schizophrenia.

3 Shahan Mamoor, MS¹

4 ¹shahanmamoor@gmail.com

5 East Islip, NY USA

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 type 2 deiodinase (D2), Dio2, in neurons of the dorsolateral pre-frontal cortex
10 from patients with schizophrenia and schizoaffective disorder.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

27 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
28 biology of schizophrenia, Dio2.

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^{2,3} 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. *Dio2* was among the genes most differentially expressed in two cell types of the brain in patients with psychotic disorders.

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.
6
7

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 Dio2 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).
16
17
18
19

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.
24
25

26 Dio2 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral pre-
27 frontal cortex in patients with schizophrenia and schizoaffective disorder.
28

1 We observed differential expression of the type 2 deiodinase, D2, encoded by the gene
2 Dio2, in the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in patients
3 with schizophrenia and schizoaffective disorder². When sorting each of the transcripts whose
4 expression was measured by microarray based on significance of change in expression between
5 diseased and control patients, in L3 PV+ DLPFC neurons from patients with schizophrenia, Dio2
6 ranked 166 out of 49386 total transcripts. Differential expression of Dio2 was statistically
7 significant (Table 1; $p=1.07E-04$).

8
9
10
11 Dio2 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal
12 cortex in patients with schizophrenia.

13
14 We also observed differential expression of Dio2 in the layer 3 and layer 5 (L5)
15 pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the
16 transcripts whose expression was measured by microarray based on significance of change in
17 expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC
18 from patients with schizophrenia, Dio2 ranked 818 out of 49386 total transcripts (Table 3),
19 equating to differential expression greater than 98.4% of all transcripts whose expression was
20 detected and measured. Differential expression of Dio2 in L3 and L5 pyramidal neurons of the
21 DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=1.14E-03$).

22
23
24
25 Dio2 expression is significantly higher in layer 3 parvalbumin-positive neurons of the
26 dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

27 We obtained exact mRNA expression levels for the differentially expressed Dio2
28

transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare Dio2 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in Dio2 expression was statistically significant. Dio2 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 1.111 ± 0.186 in Dio2 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 Dio2 in two types of neuronal cell types in patients with schizophrenia; Dio2 expression was significantly higher in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

Discussion

The type 2 deiodinase (D2), a selenoenzyme encoded by the Dio2 gene, functions in activation of T4 (thyroxine) to T3 (3,3',5-triiodothyroxine) through 5'-deiodination of T4^{6,7}. D2 is expressed in the pituitary gland, the brain, the reproductive tract and in the brown adipose tissue (BAT)⁸. D2 knockout mice are largely similar to wild-type except for mild growth retardation in males, and elevation of serum T4 and TSH levels despite unchanged T3 levels⁸. In addition, D3 activity in the cerebrum of D2 knock-out mice is double that of the wild-type

cerebrum⁹. There are other context-dependent changes observed in D2 knock-out mice. In wild-type mice, exposure to cold temperatures results in approximately 4-fold sympathetic activation of BAT, while in D2 knock-out mice, BAT sympathetic activation is nearly 10-fold⁹. This is associated with increased mRNA levels of uncoupling protein 1 and the peroxisome proliferator-activated receptor gamma coactivator 1, and an enhanced adrenergic response to BAT lipogenesis resulting from 24 to 48 hours of cold exposure⁹. Thus, D2 has important functions in metabolism of thyroid hormone and associated homeostasis and in physiologic responses to temperature change.

D2 is implicated in biology of the brain in multiple organisms in nature. In the Japanese quail, exposure to light results in induction of D2 expression in the mediobasal hypothalamus (MBH)¹⁰, a region of the brain important for photoperiodic time measurement, a mechanism by which mammals and other organisms can measure length of the day and change in day length to modulate physiological processes such as mating^{11,12}. As D2 functions to convert T4 to T3, direct intracerebroventricular administration of T3 can phenocopy the photoperiodic response, and inhibition of Dio2 function with the inhibitor iopanoic acid can prevent growth of the gonads¹⁰. D2 can function as a cargo protein in endoplasmic reticulum Golgi intermediary compartment (ERGIC) vesicles, and mice with an polymorphism at position 92 of D2 that changes threonine to alanine (Ala92-D2) activate the unfolded protein response, display hypothyroidism, sleep longer periods of time, engage in less physical activity and require longer periods of time for object memorization¹³. Ala92-D2 protein accumulates in the trans-Golgi

1 network and generates decreased levels of T3¹³. In humans, mutation in the transporter of
2 thyroid hormone, the monocarboxylate transporter 8 (MCT8)¹⁴ results in psychomotor defects
3 humans but not in mice due to compensation involving DIO2. Mice deficient for both DIO2 and
4 MCT8 display hypothyroidism in the periphery and the brain, in particular in the striatum, as
5 well as impairment of motor skills¹⁵. In an iodine-deficient environment in China, single
6 nucleotide polymorphisms in the DIO2 gene are significantly associated with mental
7 retardation¹⁶. In the broader population, the threonine 92 alanine polymorphism (Thr92AlaD2)
8 polymorphism is associated with a 1.3-fold increased risk of developing Alzheimer's Disease,
9 and in African Americans but not European Americans, Thr92AlaD2 is associated with a 1.33-
10 fold increased risk of dementia as well as a 1.35-fold increased risk of non-demented cognitive
11 impairment¹⁷. The CC genotype of the Thr92AlaD2 polymorphism is found at lower rates in
12 patients with depression than in healthy patients, suggesting it might function in a protective
13 manner against major depression¹⁸. Thus, genetic variation in D2 is associated with the risk of
14 developing multiple diseases of the brain including Alzheimer's Disease and major depression,
15 and in birds and in mice, D2 has important influence over processes like photoperiodic time
16 measurement, memory and sleep.

23 We found by mining independently published microarray data that the gene encoding the
24 type 2 deiodinase (D2), Dio2, was among the genes most differentially expressed in two
25 neuronal cell types from patients with schizophrenia. Dio2 may be relevant to the biology of
26 psychotic disorders including schizophrenia and schizoaffective disorder.

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. Davey, J.C., Becker, K.B., Schneider, M.J., Germain, D.L.S. and Galton, V.A., 1995. Cloning of a cDNA for the type II iodothyronine deiodinase. *Journal of Biological Chemistry*, 270(45), pp.26786-26789.
7. Germain, D.L.S. and Galton, V.A., 1997. The deiodinase family of selenoproteins. *Thyroid*, 7(4), pp.655-668.
8. Schneider, M.J., Fiering, S.N., Pallud, S.E., Parlow, A.F., St. Germain, D.L. and Galton, V.A., 2001. Targeted disruption of the type 2 selenodeiodinase gene (DIO 2) results in a phenotype of pituitary resistance to T4. *Molecular endocrinology*, 15(12), pp.2137-2148.
9. Christoffolete, M.A., Linardi, C.C., De Jesus, L., Ebina, K.N., Carvalho, S.D., Ribeiro, M.O., Rabelo, R., Curcio, C., Martins, L., Kimura, E.T. and Bianco, A.C., 2004. Mice with targeted disruption of the Dio2 gene have cold-induced overexpression of the uncoupling protein 1 gene but fail to increase brown adipose tissue lipogenesis and adaptive thermogenesis. *Diabetes*, 53(3), pp.577-584.
10. Yoshimura, T., Yasuo, S., Watanabe, M., Iigo, M., Yamamura, T., Hirunagi, K. and Ebihara, S., 2003. Light-induced hormone conversion of T 4 to T 3 regulates photoperiodic response of gonads in birds. *Nature*, 426(6963), pp.178-181.

- 11.Elliott, J.A., 1976, October. Circadian rhythms and photoperiodic time measurement in mammals. In *Federation proceedings* (Vol. 35, No. 12, pp. 2339-2346).
- 12.Goldman, B.D., 2001. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *Journal of biological rhythms*, 16(4), pp.283-301.
- 13.Jo, S., Fonseca, T.L., Bocco, B.M., Fernandes, G.W., McAninch, E.A., Bolin, A.P., Da Conceição, R.R., Werneck-de-Castro, J.P., Ignacio, D.L., Egri, P. and Németh, D., 2019. Type 2 deiodinase polymorphism causes ER stress and hypothyroidism in the brain. *The Journal of clinical investigation*, 129(1), pp.230-245.
- 14.Jansen, J., Friesema, E.C., Kester, M.H., Schwartz, C.E. and Visser, T.J., 2008. Genotype-phenotype relationship in patients with mutations in thyroid hormone transporter MCT8. *Endocrinology*, 149(5), pp.2184-2190.
- 15.Bárez-López, S., Grijota-Martínez, C., Ausó, E., Fernández-de Frutos, M., Montero-Pedrazuela, A. and Guadaño-Ferraz, A., 2019. Adult mice lacking Mct8 and Dio2 proteins present alterations in peripheral thyroid hormone levels and severe brain and motor skill impairments. *Thyroid*, 29(11), pp.1669-1682.
- 16.Guo, T.W., Zhang, F.C., Yang, M.S., Gao, X.C., Bian, L., Duan, S.W., Zheng, Z.J., Gao, J.J., Wang, H., Li, R.L. and Feng, G.Y., 2004. Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *Journal of medical genetics*, 41(8), pp.585-590.
- 17.McAninch, E.A., Rajan, K.B., Evans, D.A., Jo, S., Chaker, L., Peeters, R.P., Bennett, D.A., Mash, D.C. and Bianco, A.C., 2018. A common DIO2 polymorphism and Alzheimer disease dementia in African and European Americans. *The Journal of Clinical Endocrinology & Metabolism*, 103(5), pp.1818-1826.
- 18.Gałecka, E., Talarowska, M., Orzechowska, A., Górski, P., Bieńkiewicz, M. and Szemraj, J., 2015. Association of the DIO2 gene single nucleotide polymorphisms with recurrent depressive disorder. *Acta Biochimica Polonica*, 62(2).

Rank	ID	p-value	t	B	FC	Gene
166	11721579_a_at	1.07E-04	3.99	1.095454	1.111 ± 0.186	DIO2

Table 1: Dio2 is differentially expressed in the layer 3 PV+ neurons of the dorsolateral prefrontal 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 Dio2 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
818	11721579_a_at	1.14E-03	3.3237156	-0.98146	DIO2

Table 2: Dio2 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 and gene are listed in this chart.

Figure 1: Dio2 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 Dio2 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 Dio2 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.