PAX8 is differentially expressed in the brains of patients with psychotic disorders.

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We used public and published microarray data^{1,2} to identify the most significant gene expression changes in the brains of patients with psychotic disorders. We identified the paired box-containing transcription factor PAX8 as differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder. In neurons of the dorsolateral prefrontal cortex, PAX8 was expressed at significantly higher levels in patients with psychotic disorders.

Keywords: schizophrenia, psychotic disorders, systems biology of schizophrenia, psychosis, PAX8.

Psychotic disorders including schizophrenia and schizoaffective disorder are characterized by the presence of psychosis, or an inability to engage in reality testing^{3,4}. Patients with psychotic disorders can experience positive as well as negative symptoms⁵. Positive symptoms include hallucinations, visual and auditory, as well as delusions, or fixed false beliefs⁵. Negative symptoms include anhedonia, or an inability to experience pleasure, as well as avolition, or loss of motivation⁵. To understand how specific regions of the brain differ most significantly in terms of gene expression in patients with psychotic disorders, we used published and public microarray data to compare global gene expression profiles of brain tissues from patients with psychotic disorders with that of control subjects^{1,2}. We found that PAX8 was among the genes whose expression changed most significantly in patients with schizophrenia in the dorsolateral prefrontal cortex, the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and in the layer 3 and layer 5 pyramidal neurons of the DLPFC in patients with schizophrenia. PAX8 may be relevant for future study of genes pertinent to the biology of psychotic disorders.

Methods

We used microarray datasets GSE93577¹ and GSE87610² for this differential gene expression analysis in conjunction with GEO2R. GSE93577 was generated using Affymetrix Human Genome U219 Array technology with $n=68$ for control subjects and $n=70$ for patients with schizophrenia and schizoaffective disorders, using layer 3 (L3) parvalbumin neurons of the

dorsolateral prefrontal cortex (DLPFC). GSE87610 was generated using Affymetrix Human Genome U219 technology with $n=72$ for control subjects and $n=65$ for patients with schizophrenia, using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether PAX8 expression was significantly different when comparing neurons from the DLPFC from control subjects and or from patients with schizophrenia or schizophrenia and schizoaffective disorder, respectively, using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published and public microarray datasets to identify differentially expressed genes in the brains of patients with psychotic disorders.

PAX8 is differentially expressed in the PV+ L3 neurons dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder.

We identified PAX8 as among the genes whose expression changed most significantly genome-wide when comparing the transcriptional profiles of parvalbumin-positive L3 neurons of the dorsolateral prefrontal cortex (DLPFC) from control subjects and from patients with schizophrenia or schizoaffective disorder (Table 1). When sorting each of the

transcripts identified by microarray based on degree of change in expression between control subjects and patients with psychotic disorders, PAX8 ranked 216 out of 49386 total transcripts (Table 1). Differential expression of PAX8 in the PV+ L3 neurons of the DLPFC of patients with psychotic disorders in this second dataset was statistically significant (Table 1; $p=1.48E-04$).

PAX8 is differentially expressed in the L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex in patients with schizophrenia.

We mined a second microarray dataset to determine if differential expression of PAX8 in patients with psychotic disorders could be observed. We found here that PAX8 was among the genes most differentially expressed when comparing L3 and L5 pyramidal neurons from patients with schizophrenia to that of normal subjects. When sorting each of the transcripts identified by microarray based on degree of change in expression between control subjects and patients with schizophrenia, PAX8 ranked 4084 out of 49386 total transcripts (Table 2). Differential expression of PAX8 in the DLPFC of patients with schizophrenia was statistically significant (Table 2; $p=2.08E-02$).

PAX8 expression is significantly higher in the PV+ L3 neurons dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder.

We obtained exact mRNA expression levels for the differentially expressed PAX8 transcript, from control subjects and from patients with schizophrenia, to directly compare PAX8 expression levels in the PV+ L3 neurons of the DLPFC. We also performed a statistical test to

determine if this difference in PAX8 expression was statistically significant. PAX8 was expressed at higher levels in the PV+ L3 neurons of the DLPFC in patients with schizophrenia, and this difference was statistically significant (Figure 1; $p=0.0001$). We calculated a mean fold change of 1.0489 ± 0.0742 in PAX8 expression when comparing substantia nigra from patients with schizophrenia to that of control subjects (Table 1).

Thus, we found that PAX8 was among the most differentially expressed genes in the brains of patients with psychotic disorders, in parvalbumin-positive L3 neurons and in L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex; PAX8 expression was significantly higher in PV+ L3 neurons of the DLPFC in patients with schizophrenia.

Discussion

Pax8 is a paired box-containing transcription factor that was originally described in the context of binding to thyroid-specific promoters of thyroglobulin and thyroperoxidase, genes with exclusive expression in the thyroid⁶⁻⁹. Pax8 binding sites display overlap with that of binding sites for the transcriptional factor TTF-1 and can cooperate with TTF-1 in transactivation of thyroglobulin, thyroperoxidase and the sodium/iodide symporter (NIS)⁶. The thyroid is composed of two major cells types; follicular cells of endodermal origin and parafollicular C-cells of neural crest origin. While mice lacking TTF-1 lack both cell types, mice deficient in Pax8 are specifically lacking in follicular cells; thus, Pax8 is required for the development of endodermal lineage cells of the thyroid into follicular cells⁸. In the brain, Pax8 expression can be

1 detected between embryonic days 11.5 and 12.5 from the myelencephalon through the neural
2 tube, along the rostrocaudal axis⁶. This expression is lost by E13.5⁶. Pax8 is also important for
3 development of the kidney¹⁰. Mammalian kidney development proceeds from the pronephros to
4 the mesonephros to the adult pronephros; the embryos of mice lacking both Pax2 and Pax8 are
5 unable to form nephric ducts¹⁰. Homozygous Pax8 deficient mice are born in normal Mendelian
6 ratio but die around weaning age due to failure of thyroid development¹⁰. In Pax8 +/- Pax2 +/-
7 mice, the number and arrangement of nephric tubules and glomeruli were abnormal; tubule
8 induction appeared inefficient as there were increased amounts of uninduced mesenchyme at the
9 cortex as well as uninduced mesenchymal stroma¹⁰. Kidneys in Pax8 +/- Pax2 +/- heterozygous
10 double mutants were 25% the size of wild-type kidneys¹⁰. Interestingly, 100% of Pax8 +/-
11 Pax2 +/- C57BL/6x129/Sv mice displayed vaginal atresia, while only approximately 43% of
12 Pax8 +/- Pax2 +/- C3H/He females developed vagina atresia, indicating that factors specific to the
13 animals genetic background were important in penetrance of this phenotype. There was a
14 marked absence of mesenchymal-epithelial transition in the intermediate mesoderm of Pax8 -/-
15 Pax2 -/- embryos, a hallmark of the onset of pronephros formation¹⁰.

16 While the ears of Pax8 -/- Pax2 -/- mice do not develop past the otocyst stage, Pax8 -/-
17 mice are phenotypically normal indicating that Pax2 can compensate for Pax8 in this context¹¹.
18 Thus, Pax8 is important for the development of the thyroid and the kidneys; while Pax2 Pax8
19 double deficient mice manifest an inner ear phenotype, this appears to be attributable to
20 functions of Pax2 and not Pax8¹¹.

1 There are limited reports of Pax8 involvement in human disease. In human cancers, Pax8
2 can be found as a gene fusion to PPAR γ 1 as a result of a t(2;3)(q13;p25) translocation. PAX8-
3 PPAR γ 1 can function as an oncogene in carcinoma of the thyroid and inhibit PPAR γ 1
4 transactivation induced by thiazolidinediones in a dominant negative fashion¹². Mutations in the
5 paired-box domain of Pax8 are found in congenital hypothyroidism; these mutations
6 significantly impair Pax8 DNA-binding¹³. The transcription factor sharing overlap with Pax8 in
7 the thyroid, TTF1 (Nkx2-1), is mutated in brain-lung-thyroid syndrome, characterized by the
8 presence of congenital hypothyroidism, infant respiratory distress syndrome and benign
9 hereditary chorea. Deficient transactivation of a P202L Nkx2-1 mutant found in brain-lung-
10 thyroid syndrome could be rescued by ectopic expression of Pax8, indicating that Pax8 could
11 compensate at the transcriptional level in the context of a human disease involving the brain. In
12 the brain, Nkx2-1 expression can be found in as early as day 32 in the prosencephalon as well as
13 in hypothalamic neurons¹⁴. We could not identify further information describing roles for Pax8
14 in biology of the brain or in human diseases of the brain.

20 We observed significant changes in gene expression of PAX8 in the dorsolateral
21 prefrontal cortex of patients with psychotic disorders, in L3 PV+ neurons and in L3 and L5
22 pyramidal neurons. If validated in separate cohorts of patients with schizophrenia or
23 schizoaffective disorder, the phenotypic consequence(s) of graded changes in PAX8 gene
24 expression can be evaluated in wild-type neurons *in vitro* or in neurons generated from
25 fibroblasts of patients with schizophrenia. PAX8 is expressed in the neural tube during
26 development⁶ and may be of relevance to the biology of schizophrenia.

References

1. GSE93577. Enwright J.F., Huo Z, Arion D, Corradi J.P, Tseng G., and Lewis D.A. University of Pittsburgh, Psychiatry and Neuroscience. Pittsburgh, PA. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE93577>
2. 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.
3. Cannon, T.D., 2015. How schizophrenia develops: cognitive and brain mechanisms underlying onset of psychosis. *Trends in cognitive sciences*, 19(12), pp.744-756.
4. Arciniegas, D.B., 2015. Psychosis. *Continuum: Lifelong Learning in Neurology*, 21(3 Behavioral Neurology and Neuropsychiatry), p.715.
5. 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.
6. Plachov DI, Chowdhury KA, Walther CL, Simon DO, Guenet JL, Gruss PE. Pax8, a murine paired box gene expressed in the developing excretory system and thyroid gland. *Development*. 1990 Oct 1;110(2):643-51.
7. Zannini, M., Francis-Lang, H., Plachov, D. and Di Lauro, R., 1992. Pax-8, a paired domain-containing protein, binds to a sequence overlapping the recognition site of a homeodomain and activates transcription from two thyroid-specific promoters. *Molecular and Cellular Biology*, 12(9), pp.4230-4241.
8. Mansouri, A., Chowdhury, K. and Gruss, P., 1998. Follicular cells of the thyroid gland require Pax8 gene function. *Nature genetics*, 19(1), pp.87-90.
9. di Magliano, M.P., Di Lauro, R. and Zannini, M., 2000. Pax8 has a key role in thyroid cell differentiation. *Proceedings of the National Academy of Sciences*, 97(24), pp.13144-13149.
10. Bouchard, M., Souabni, A., Mandler, M., Neubüser, A. and Busslinger, M., 2002. Nephric lineage specification by Pax2 and Pax8. *Genes & development*, 16(22), pp.2958-2970.

11. Bouchard, M., de Caprona, D., Busslinger, M., Xu, P. and Fritzsch, B., 2010. Pax2 and Pax8 cooperate in mouse inner ear morphogenesis and innervation. *BMC developmental biology*, 10(1), p.89.
12. Kroll, T.G., Sarraf, P., Pecciarini, L., Chen, C.J., Mueller, E., Spiegelman, B.M. and Fletcher, J.A., 2000. PAX8-PPAR γ 1 fusion in oncogene human thyroid carcinoma. *Science*, 289(5483), pp.1357-1360.
13. Macchia, P.E., Lapi, P., Krude, H., Pirro, M.T., Missero, C., Chiovato, L., Souabni, A., Baserga, M., Tassi, V., Pinchera, A. and Fenzi, G., 1998. PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. *Nature genetics*, 19(1), pp.83-86.
14. Carré, A., Szinnai, G., Castanet, M., Sura-Trueba, S., Tron, E., Broutin-L'Hermite, I., Barat, P., Goizet, C., Lacombe, D., Moutard, M.L. and Raybaud, C., 2009. Five new TTF1/NKX2.1 mutations in brain–lung–thyroid syndrome: rescue by PAX8 synergism in one case. *Human molecular genetics*, 18(12), pp.2266-2276.

Rank	ID	p-value	t	B	FC	Gene
216	11741652_a_at	1.48E-04	3.90	0.814318	1.0489 ± 0.0742	PAX8

Table 1: PAX8 is differentially expressed in L3 parvalbumin neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

The rank of differential expression, the probe/transcript 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 PAX8 in neurons of the DLPFC when compared to DLPFC neurons from control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
4084	11741652_a_at	2.08E-02	2.3383801	-3.46142	PAX8

Table 2: PAX8 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex in patients with schizophrenia.

The rank of differential expression, the probe/transcript 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 PAX8 in neurons of the DLPFC when compared to DLPFC neurons from control subjects, the gene and gene name are listed in this chart.

Figure 1: PAX8 is expressed at significantly higher levels in the L3 PV+ neurons of the DLPFC in patients with schizophrenia and schizoaffective disorder.

The mRNA expression of PAX8 in L3 PV+ DLPFC neurons from control subjects (left) and in the L3 PV+ DLPFC neurons from patients with schizophrenia or schizoaffective disorder (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in PAX8 expression between unaffected L3 PV+ neurons of the DLPFC and L3 PV+ neurons of the DLPFC from patients with psychotic disorders, a *p*-value, listed above.