Imprinted gene in the Prader-Willi syndrome region (IPW) is differentially expressed in the brains of patients with psychotic disorders.

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We used published and public microarray data (1, 2) to identify the most significant gene expression changes in the brains of patients with psychotic disorders. We identified Imprinted gene in the Prader-Willi syndrome region (IPW) (3) as differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia as well as in the parvalbumin-positive layer 3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder. IPW likely encodes a non-coding RNA (4, 5) and is a regulator of the imprinted DLK1-DIO3 locus (4); we found that IPW was expressed at significantly higher levels in the dorsolateral prefrontal cortex of patients with psychotic disorders.

Keywords: schizophrenia, psychotic disorders, systems biology of schizophrenia, psychosis, non-coding RNAs in human disease.

Psychotic disorders including schizophrenia and schizoaffective disorder are characterized by the presence of psychosis, or an inability to engage in reality testing (6, 7). Patients with psychotic disorders can experience positive as well as negative symptoms (8). Positive symptoms include hallucinations, visual and auditory, as well as delusions, or fixed false beliefs (8). Negative symptoms include anhedonia, or an inability to experience pleasure, as well as avolition, or loss of motivation (8). 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 non-affected, control subjects (1, 2). We found that IPW was among the genes whose expression changed most significantly in patients with schizophrenia in the dorsolateral prefrontal cortex, as well as in the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder. IPW may be relevant for future study of genes pertinent to the biology of psychotic disorders.

Methods

We used microarray datasets GSE87610 (1) and GSE93577 (2) for this differential gene expression analysis in conjunction with GEO2R. GSE87610 was generated using Affymetrix Human Genome U219 with n=72 for control subjects and n=65 for patients with schizophrenia. 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. 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 IPW expression was significantly different when comparing normal DLPFC

tissue from control subjects and DLPFC or L3 PV+ neurons of the DLPFC from patients with schizophrenia or schizophrenia and schizoaffective disorder, respectively, using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined microarray datasets, published (1) and public (2), to identify genes whose expression was most different in the brains of patients with psychotic disorders when compared to control subjects.

IPW is differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia.

We identified IPW as among the genes whose mRNA expression was most different in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects (1). When sorting all of the genes expressed in the dorsolateral prefrontal cortex, as measured by microarray, based on change in expression between psychotic patients and control subjects, IPW ranked 14 out of 12188 total transcripts. Differential expression of IPW in the dorsolateral prefrontal cortex of patients with schizophrenia was statistically significant (Table 1; $p=3.04E-08$).

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

In a separate dataset, we identified IPW as differentially expressed in the parvalbumin (PV)-positive L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder when compared to control subjects (2). When sorting all of the genes expressed in PV+ L3 neurons of the dorsolateral prefrontal cortex, as measured by microarray, based on change in expression between patients with schizophrenia or schizoaffective disorder and control subjects, IPW ranked 189 out of 49386 total transcripts. Differential expression of

IPW in the PV+ L3 neurons of the DLPFC of patients with schizophrenia or schizoaffective disorder was statistically significant (Table 2; $p=1.28E-04$).

IPW expression is significantly higher in the dorsolateral prefrontal cortex of patients with schizophrenia.

Next we obtained exact mRNA expression levels of IPW from the dorsolateral prefrontal cortex of patients with schizophrenia and from the dorsolateral prefrontal cortex of control subjects to directly compare IPW expression between these groups. We also performed a statistical test to evaluate whether this difference in expression was statistically significant. IPW was expressed at significantly higher levels in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects (Figure 1; $p<0.0001$). We calculated a 1.0976 ± 0.0912 fold change in IPW expression in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects (Table 1).

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

We also obtained exact mRNA expression levels of IPW from PV+ L3 neurons of the dorsolateral prefrontal cortex from patients with schizophrenia or schizoaffective disorder and from the dorsolateral prefrontal cortex of control subjects to directly compare IPW between these two groups. We also performed a statistical test to evaluate whether this difference in expression was statistically significant. IPW was expressed at significantly higher levels in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder when compared to control subjects (Figure 2; $p=0.0002$). We calculated a 1.0327 ± 0.0395 fold change in IPW expression in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder when compared to control subjects (Table 2).

Thus, we identified using three public and published microarray datasets (2-4) that IPW was among the genes most differentially expressed in the brain tissues of patients with psychotic disorders, in the dorsolateral prefrontal cortex and in the PV+ L3 neurons of the dorsolateral prefrontal cortex. Thus, a putative non-coding RNA encoded by a paternally imprinted gene is among the transcripts whose expression is most quantitatively different in the dorsolateral prefrontal cortex of patients with schizophrenia and schizoaffective disorder.

Discussion

We compared transcriptome information from published and public microarray datasets generated using the brain tissues of patients with psychotic disorders, specifically, in the dorsolateral prefrontal cortex, to that of non-affected control subjects, to identify the most significant changes in gene expression in the brains of patients with psychotic disorders. We found that changes in IPW gene expression were more significant than greater than 99% of transcriptome in group of patients.

IPW is a gene encoded at an imprinted, paternally expressed locus at 15q11-q13 (3). In mice, IPW is expressed in the brain in adults as well as during development (9, 10). In cattle, IPW is expressed in the brain, heart, kidney, liver, lung, spleen, and skeletal muscle (5). Patients with Prader-Willi Syndrome possess genomic alterations at 15q11-q13 in the form of *de novo* deletions, maternal uniparental disomy, or imprinting defects - deletions of a unique genetic element known as the imprinting center. (11). These alterations lead to loss of expression of imprinted paternally expressed genes at 15q11-q13, one of which is IPW (11). IPW likely encodes a non-coding RNA (4, 5); none of the IPW transcripts produced in cattle contain Kozak consensus sequences (5). The IPW may actually be one extended transcript representing the host gene for a series of tandemly repeated, imprinted snoRNAs (11).

1 One study demonstrated that IPW non-coding RNA can regulate gene expression at the
2 DLK1-DIO3 imprinted locus (4). DLK1-DIO3 is a genomic region that contains maternally
3 imprinted genes including MEG3 (Gtl2) MEG8 (RIAN), as well as paternally imprinted genes
4 including DLK1, RTL1, and DIO3. Moreover, the DLK1-DIO3 region possesses a microRNA
5 “cluster” encoding 54 microRNAs (11). Thus, an imprinted paternally expressed gene can
6 regulate the expression of a separate imprinted locus containing both maternally imprinted and
7 paternally imprinted genes; this locus possesses a multitude of microRNAs, each of which can
8 modulate the expression of a network of genes. We found using published and public
9 microarray data that patients with psychotic disorders express significantly higher levels of IPW
10 in the dorsolateral prefrontal cortex than non-affected control subjects, and that this difference
11 in IPW expression was more significant than the difference in gene expression of most of the
12 transcriptome. It is of interest to determine experimentally whether changes in IPW expression
13 in the dorsolateral prefrontal cortex of patients with schizophrenia, if validated, translate to
14 differences in gene expression at the DLK1-DIO3 imprinted locus.
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Rank	ID	p-value	t	B	Fold change	Gene
14	11756264_s_at	3.04E-08	5.8759646	8.46292	1.0976 ± 0.0912	IPW

Table 1: IPW is differentially expressed in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects.

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 IPW in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene
189	11756264_s_at	1.28E-04	3.94	0.940848	1.0327 ± 0.0395	IPW

Table 2: IPW is differentially expressed in PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder when compared to control subjects.

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 IPW in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: IPW is expressed at significantly higher levels in the dorsolateral prefrontal cortex of patients with schizophrenia when compared to control subjects.

The mRNA expression of IPW in the dorsolateral prefrontal cortex of control subjects (left) and in the dorsolateral prefrontal cortex of patients with schizophrenia (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in IPW expression between the dorsolateral prefrontal cortex of control subjects and patients with schizophrenia, a p-value, listed above.

Figure 2: IPW is expressed at significantly higher levels in the PV+ L3 neurons of the dorsolateral prefrontal cortex of patients with schizophrenia or schizoaffective disorder than in the PV+ L3 neurons of the DLPFC of control subjects.

The mRNA expression of IPW in the PV+ L3 neurons of the dorsolateral prefrontal cortex of control subjects (left) and in the PV+ L3 neurons of the dorsolateral prefrontal cortex of 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 IPW expression between the PV+ L3 neurons of the dorsolateral prefrontal cortex of control subjects and patients with schizophrenia or schizoaffective disorder, a p-value, listed above.