NDUFA13/YJEFN3 is differentially expressed in the brains of patients with schizophrenia.

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Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined published and public microarray datasets^{2,3} to discover differentially expressed genes in schizophrenia and schizoaffective disorder. We found significant differential expression of transcripts overlapping NDUFA13 and YJEFN3 genes in neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder.

Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems biology of schizophrenia, NDUFA13, YJEFN3.

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. *NDUFA13/YJEFN3* were 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 NDUFA13/YJEFN3 expression was significantly different when comparing neurons of
13 the DLPFC from control subjects and from patients with schizophrenia and schizoaffective
14 disorder using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all
15 statistical analyses (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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26 NDUFA13/YJEFN3 is differentially expressed in layer 3 parvalbumin-positive neurons of the
27 dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.
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We observed differential expression of a transcript detected by a probe corresponding to a region overlapping both NDUFA13 and YJEFN3 genes 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, NDUFA13/YJEFN3 ranked 1 out of 49386 total transcripts, equating to 99.998% differential expression. Differential expression of NDUFA13/YJEFN3 was statistically significant (Table 1; $p=1.12E-08$).

NDUFA13/YJEFN3 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

Intriguingly, a NDUFA13/YJEFN3 transcript was also among the most differentially expressed in a separate dataset, 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, NDUFA13/YJEFN3 ranked 18 out of 49386 total transcripts (Table 2), equating to 99.96% differential expression. Differential expression of NDUFA13/YJEFN3 in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=1.33E-07$).

NDUFA13/YJEFN3 expression is significantly lower 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 NDUFA13/YJEFN3 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare NDUFA13/YJEFN3 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in NDUFA13/YJEFN3 expression was statistically significant. NDUFA13/YJEFN3 was expressed at lower 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 0.9 ± 0.1 in NDUFA13/YJEFN3 expression when comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder to that of control subjects (Table 1).

NDUFA13/YJEFN3 expression is significantly lower in L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

We obtained exact mRNA expression levels for the differentially expressed NDUFA13/YJEFN3 transcript in L3 and L5 pyramidal neurons from control subjects and from patients with schizophrenia, to directly compare NDUFA13/YJEFN3 expression levels in L3 and L5 pyramidal neurons of the DLPFC. We also performed a statistical test to determine if this difference in NDUFA13/YJEFN3 expression was statistically significant. NDUFA13/YJEFN3

was expressed at lower levels in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia, and this difference was statistically significant (Figure 2; $p < 0.0001$). We calculated a mean fold change of 0.9389 ± 0.0724 in NDUFA13/YJEFN3 expression when comparing L3 and L5 pyramidal neurons from patients with schizophrenia to that of control subjects (Table 2).

Thus, we found significant differential expression of NDUFA13/YJEFN3 in two types of neuronal cell types in patients with schizophrenia; NDUFA13/YJEFN3 expression was significantly lower in both L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder and in L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex in patients with schizophrenia.

Discussion

NDUFA13 is an accessory subunit of the mitochondrial complex I. Also known as GRIM-19 and B16.6, NDUFA13 is interferon-beta and retinoic acid inducible⁵. NDUFA13 is found in both I α and I λ sub-complexes of complex I⁶. It has been suggested that the position of NDUFA13 alludes to possible channel function of NDUFA13, with a 63-residue, membrane-spanning helix interacting with the amino terminus of NDUF8 and anchored under a “heel”⁶. Mice with a cardiac-specific heterozygous NDUFA13 knockout mice (cHet) display increased resistance to apoptosis in response to ischemia-reperfusion injury⁷. In the cytosol but not in the mitochondria, NDUFA13 cHet mice contain increased hydrogen peroxide levels due to leakage of complex I, leading to dimerization of STAT3 and resulting in decreased infarct size as STAT3 dimerization decreased superoxide burst by activation of anti-apoptotic signal transduction

1 accompanied by cleavage of caspase-3⁷. Germline mutation of NDUFA13 has been found in two
2 patients, sisters, with dyskinesia, sensory deficiencies and early onset hypotonia; muscle and
3 fibroblasts from these patients possess reduced complex I activity, supporting NDUFA13
4 function in cellular respiration⁸. Another case report documents a patient with Leigh Syndrome
5 lesions in the brain, with hypotonia, nystagmus and spastic tetraparesis; next-generation
6 sequencing identified biallelic NDUFA13 mutations⁹. This patients fibroblasts were also
7 deficient in complex I function as measured by basal oxygen consumption rate, maximum
8 oxygen consumption rate and ATP production, indicating defects in oxidative phosphorylation⁹.
9 Thus, in humans, a disease of the central nervous system can result from NDUFA13 mutation.
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12 YJEFN3 (hYjeF_N3) was identified in a screen for proteins containing the YjeF_N
13 domain found in the ApoA-I-binding protein (AI-BP)¹⁰. YJEFN3 is expressed in the brain, the
14 mammary glands, the ovaries and the testes¹⁰. YJEFN3 is expressed in the Leydig cells of the
15 testes and the theca cells of the ovaries suggesting a possible function of YJEFN3 in metabolism
16 of steroid hormones¹⁰. In a genome-wide association study searching for single-nucleotide
17 polymorphisms (SNPs) conferring risk or protection from schizophrenia, linkage disequilibrium
18 indicated that, while not the primary gene related to the SNP in question, YJEFN3 resides within
19 one genomic region with significant association with schizophrenia¹¹.
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24 We discovered differential expression of transcripts detected by a probe corresponding to
25 a region of the genome overlapping both NDUFA13 and YJEFN3 genes (“NDUFA13/
26 YJEFN3”). These transcripts were among the most differentially expressed, transcriptome-wide,
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1 in two neuronal cell types of the dorsolateral prefrontal cortex in patients with schizophrenia: the
2 layer 3 PV+ neurons and the layer 3 and layer 5 pyramidal neurons. Differential expression of
3 NDUFA13/YJEFN3 should be validated in separate and larger schizophrenia patient datasets.
4 NDUFA13/YJEFN3 may be of relevance to the cell biology of the brain in schizophrenia and
5 associated psychoses: visual and auditory hallucinations.
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Rank	ID	p-value	t	B	FC	Gene
1	11757719_x_at	1.12E-08	-6.08	9.231797	0.9 ± 0.1	NDUFA13 /// YJEFN3

Table 1: NDUFA13/YJEFN3 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 NDUFA13/YJEFN3 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	FC	Gene
18	11720342_s_at	1.33E-07	-5.5651024	7.12472	0.9389 ± 0.0724	NDUFA13 /// YJEFN3

Table 2: NDUFA13/YJEFN3 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: NDUFA13/YJEFN3 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 NDUFA13/YJEFN3 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 NDUFA13/YJEFN3 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.

Figure 2: NDUFA13/YJEFN3 is expressed at significantly lower levels in L3 and L5 pyramidal neurons of the dorsolateral prefrontal cortex in patients with schizophrenia.

The mRNA expression of NDUFA13/YJEFN3 in L3 and L5 pyramidal neurons from control subjects (left) and in L3 and L5 pyramidal neurons from 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 NDUFA13/YJEFN3 expression between control L3 and L5 pyramidal neurons and L3 and L5 pyramidal neurons from control subjects from patients with schizophrenia, a p-value, listed above.