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ScienceDirect
Schizophrenia Research 98 (2008) 111 – 117
SCHIZOPHRENIA RESEARCH
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# Histone deactylase 1 expression is increased in the prefrontal cortex of schizophrenia subjects: Analysis of the National Brain Databank microarray collection ☆

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Received 27 June 2007; received in revised form 10 September 2007; accepted 14 September 2007
Available online 24 October 2007

**Abstract**

Histone deactylase enzymes are responsible for the deacetylation of histone tails, and consequently influence gene regulation through their ability to modify chromatin structure surrounding promoter regions. We analyzed the microarray collection of the National Brain Databank to investigate differential expression of these enzymes in the prefrontal cortices of control, schizophrenia and bipolar subjects. HDAC1 expression levels were significantly higher in schizophrenia versus normal subjects. The mRNA expression level of an epigenetically regulated schizophrenia candidate gene GAD67 was strongly and negatively correlated with the mRNA expression levels of HDAC1, HDAC3 and HDAC4 levels. These findings provide additional support for the proposal that epigenetic factors are operative in the brain pathology of patients with schizophrenia.


*Keywords:* HDAC enzymes; GAD67 expression; Schizophrenia; Postmortem brain; Microarray; Gender; Age

## 1. Introduction

Chromatin, a DNA–protein complex, commonly conceptualized as the efficient "packaging" of several billion bases of genomic DNA, functions as an interactive platform for the regulation of gene transcription. Chromatin participation in gene regulation is based on physical and chemical adaptations in the vicinity of regulatory DNA sequences, the mechanics of which are determined by linear patterns of covalent modifications of cytosine bases in DNA and amino acid residues in histone protein tails. These covalent modifications, along with their attendant enzymes and cognate regulatory proteins, are broadly classified under the general term "epigenetic mechanisms." Acetylation of site specific lysine residues along the N-terminal tail of histone 3 and histone 4 proteins is one such modification that putatively releases the histone tail from its position around the DNA strand, exposing regulatory regions and facilitating DNA–protein interactions with transcriptional regulators. Acetyl groups are covalently attached to these residues by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs). The HDAC family of enzymes (HDAC 1–11) is ubiquitously expressed in most tissues

☆ This work was presented at the Society of Biological Psychiatry meeting in San Diego, May 2007.

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doi:10.1016/j.schres.2007.09.020

including the brain (de Ruijter et al., 2003), and is widely recognized as potential therapeutic targets for brain disorders (Sharma 2005; Tsankova et al., 2006; Simonini et al., 2006,).

Epigenetic gene regulation in the brain is instrumental for neuronal viability and survival (Fan et al., 2001), membrane depolarization (Chen et al., 2003, Martinowich et al., 2003, Sharma et al., 2007), synaptic plasticity (Levenson et al., 2006), and long-term potentiation, cognition and memory-consolidation (Miller and Sweatt 2007; Alarcon et al., 2004, Korzus et al., 2004). Abnormalities in epigenetic gene regulation as a cause of complex disorders has emerged as a powerful heuristic method towards the study of molecular mechanisms in psychiatric disorders (for review Costa et al., 2004, Petronis et al., 1999, Sharma 2005). A mounting body of evidence has developed, to support abnormalities in epigenetic gene regulation of prototypic schizophrenia candidate genes such as reelin and GAD67, from cell studies (Chen et al., 2002, Noh et al., 2005, Kundakovic et al., 2006), animal investigations (Tremolizzo et al., 2005, Dong et al., 2005) and from DNA methylation and expression/protein studies in the postmortem brains of schizophrenia subjects (Akbarian et al., 2005, Grayson et al., 2005, Abdolmaleky et al., 2005, 2006, Veldic et al., 2004, 2005, 2007, Ruzicka et al., 2007, Guidotti et al., 2007, Benes et al., 2007). Extending these concepts to clinical populations, we have recently reported that schizophrenia patients may have a comparatively restrictive chromatin structure measured in their peripheral blood lymphocytes (Sharma et al., 2006), and that GAD67 is directly regulated by an inhibitor of histone deactylases in primary lymphocyte cultures (Gavin et al., 2007 submitted). These findings have motivated an investigation of diagnostic differences in the expression of histone modifying enzymes in postmortem brains that would suggest the presence of an environment conducive to the development of restrictive chromatin. Further, because of the connection between levels of HDAC activity and schizophrenia candidate genes such as GAD67 (extensively replicated down-regulation in the postmortem brains of schizophrenia patients as reviewed by Akbarian and Huang 2006), we looked for a functional association between the mRNA expression levels of HDAC enzymes and GAD67. These questions are examined using a restricted and targeted analysis of the National Brain Databank (NBD) microarray collection.

## 2. Method

The National Brain Databank is made available by the Harvard Brain Tissue Resource Center as a publicly accessible data repository for neuroscience investigators. The databank provides microarray expression results from postmortem brain tissue samples obtained from the prefrontal cortices of subjects with psychiatric and neurological illnesses. In particular, the collection includes samples from 27 control subjects, 16 schizophrenia subjects and 18 bipolar subjects as well as 3 subjects with schizoaffective disorder. Further, the collection includes 44 males and 20 females (Table 1). Thirty out of 37 patients were being treated with a psychotropic; of these all but 5 subjects (83%) were treated with multiple medications. Medications included

Table 1
Description of the National Brain Databank microarray collection

| | | Normal subjects | Schizophrenia | Bipolar disorder | Schizoaffective disorder |
|---|---|---|---|---|---|
| Age range | 20–31 | 1 | 1 | 1 | |
| | 31–40 | 7 | 2 | 3 | |
| | 41–50 | 3 | 6 | 2 | 1 |
| | 51–60 | 2 | | 1 | |
| | 61–70 | 7 | 3 | 1 | |
| | 71–80 | 5 | 3 | 8 | 1 |
| | 80+ | 2 | 1 | 2 | 1 |
| Gender | Male | 19 | 13 | 12 | |
| | Female | 8 | 3 | 6 | 3 |
| Postmortem interval | | 20.34 (5.74) | 20.27 (4.35) | 21.12 (9.73) | 24.31 (10.27) |
| Brain PH | | 6.43 (0.31) | 6.41 (0.25) | 6.45 (0.24) | 6.52 (0.35) |
| 3′/5′ G3PDH ratio | | 1.43 (0.40) | 1.58 (0.67) | 1.53 (0.48) | 1.45 (0.24) |
| 3′/5′ $\beta$-actin ratio | | 2.32 (0.86) | 2.45 (0.83) | 2.68 (1.03) | 2.19 (0.68) |
| RNA ratio | | 1.07 (0.34) | 1.12 (0.50) | 1.01 (0.32) | 0.99 (0.43) |
| Percent probe sets present | | 45.94 (3.98) | 46.1 (4.23) | 45.17 (4.76) | 44.26 (4.07) |

RNA ratios represent the ratio 28S:18S of ribosomal RNA as a measure of underlying mRNA stability. 3′/5′ expression ratios for GAPDH and β-actin reflect differential degradation at the 5′ end of the gene versus 3′ end where the polyadenylation sequence is used for hybridization to the primer oligonucleotides. ‘Percent probe sets present’ is the percent of microarray wide probes providing a signal.

typical and atypical antipsychotics, mood stabilizers (including valproic acid), antidepressants, stimulants and sedatives.

The microarray platform utilizes the Affymetrix HG-U133A gene chip. This platform represents each interrogated sequence as a set of oligonucleotides synthesized onto the chip. A collection of 22 such oligos for a given genomic sequence is called a probe set. Each probe set is comprised of 11 perfectly matched oligos and 11 mismatched oligos (by one base) — the latter serve as a type of internal standard. The abundance of a given transcript, also referred to as the 'signal' is computed by the Gene Chip Operating System (GCOS) as a one-step biweighted estimate of the combined differences of all the probe pairs in the probe set.

### *2.1. Default controls at the level of RNA quality and microarray integrity*

Four independent parameters of total RNA quality and cellular integrity (18S/28S ribosomal ratio, 3′/5′ GAPDH ratio and 3′/5′ β-actin ratio) and Genechip validity (percent probe sets present) are provided by the NBD website.

### *2.2. Scaling at the level of the total array*

For single-array analysis, GCOS software executes a mathematical scaling procedure to allow for comparisons between single array results from different experiments (subjects). This minimizes the discrepancy due to variables such as sample preparation, hybridization conditions, staining or probe array lot. The analytic software surveys signal intensity from all probes on the microarray and computes a scaling factor that scales the 'trimmed' mean signal to the user defined 'target signal'. Single arrays that are scaled to the same 'target signal' can be compared.

### *2.3. Effectiveness of individual probe design*

The HG-U133A chip can technically interrogate the expression of HDACs (1–6, 7, 9, 11). However, a given tissue such as the prefrontal cortex may not express all HDAC transcripts at an abundance level that is reliably detected by the chip. This is compounded by the additional possibility that the default probe may hybridize poorly to a tissue specific gene isoform. Among the listed HDACs, we included only those that demonstrated reliable and consistent expression in prefrontal cortex across the whole collection. In the absence of additional information, the *p*-value threshold for the Detection Call (α2) was set at 0.06 (the default value suggested by the manufacturer). If a given probe (HDAC1 or 2 etc) yielded a greater than 10% 'absent' detection call rate, this probe was not included in the analysis. This resulted in exclusion of HDAC5, HDAC7 and HDAC11. Subsequently, all 'signal' values for the remaining HDAC genes were analyzed. GAD67 was interrogated using probe ID 205278. Signal levels for HDACs (1–4, 6, 9) as well as for GAD67 were then entered into an SPSS database and analyzed for diagnostic differences and associations. Diagnostic analyses were conducted only in data from controls, schizophrenia and bipolar subjects. Parametric tests for independent groups (one-way ANOVA and independent *t*-tests) were used for group comparisons. Pearson correlations were used to establish bivariate associations. All *p*-values are two-tailed.

## 3. Results

There were no significant differences between diagnostic groups in the postmortem brain intervals, brain pHs or in values of the quality control parameters (18S/28S ribosomal ratio, 3′/5′ GAPDH ratio and 3′/5′ β-actin ratio).

In the total sample, females subjects across diagnostic groups had a significantly higher level of HDAC1 signal (312.23 vs. 247.09; $df=62$; $p<0.02$). There was no significant difference in the distribution of gender

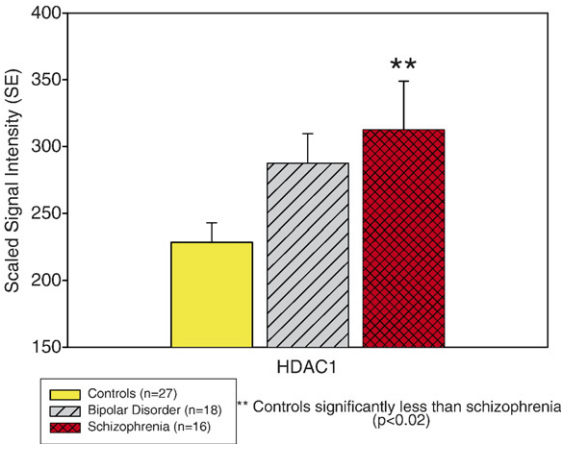


Fig. 1. A restricted analysis (on the identified genes in the text) was conducted using the microarray collection of the National Brain Databank. Expression of HDAC enzymes in samples of postmortem prefrontal cortical tissue was compared between the diagnostic groups as presented. There is a significant increase in HDAC1 expression levels in samples obtained from schizophrenia subjects compared to controls.

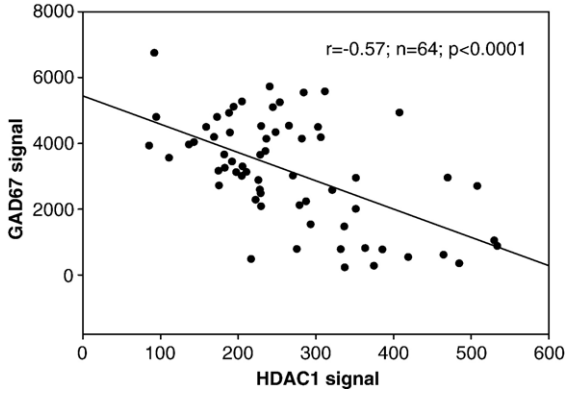


Fig. 2. GAD 67 is an epigenetically controlled gene whose regulation is influenced by the level of acetylated (increased expression) or deacetylated chromatin (decreased expression) that surrounds its promoter sequence. There was a significant correlation between HDAC1 and GAD67 expression signals in 64 subjects (controls=27, bipolar=16, schizophrenia=18, schizoaffective=3).

among the four diagnostic groups. Age was significantly related to levels of HDAC4 in a bimodal pattern, with marginally higher levels between 20 and 40 years of age, decreasing between 40 and 60 years and significantly higher ($p<0.02$) in patients above 70 years of age. All HDACs examined were equivalently expressed in either hemisphere (29 left and 35 right).

Schizophrenia patient samples had a significant increase in the mRNA expression of HDAC1 in the prefrontal cortex when compared to control samples (313.5 vs. 228.6; $p<0.02$; Fig. 1). No significant diagnostic differences were found for the expression of HDACs 2, 3, 4, 6, and 9.

GAD67 expression was significantly and negatively correlated with the expression levels of HDAC1 ($r=-0.57$; $n=64$; $p<0.0001$; Fig. 2) and HDAC3 ($r=-0.35$; $n=64$; $p<0.004$) and HDAC4 ($r=-0.41$; $n=64$; $p<0.001$) suggesting an overlapping regulation of this gene by several HDACs in the prefrontal cortices of patients with schizophrenia. Higher HDAC levels were associated with lower levels of GAD67 expression. Based on the findings of the bivariate correlations, a multiple regression with HDAC 1, 3, and 4 as independent variables and GAD67 as the dependent measure revealed a highly significant effect for the complete model ($t=8.8$; $p<9.9E-14$; and of HDAC1 by itself ($t=-4.27$; $p<0.00006$). The part correlation between HDAC1 and GAD67 was −0.41 controlling for the other independent variables (i.e., HDAC3 and HDAC4) in the model.

Because of the HDAC inhibiting properties of valproic acid, we analyzed for medication effects of valproic acid on HDAC enzyme mRNA expression in the prefrontal cortex. Patients being treated with valproic acid (8 bipolars, 1 schizophrenia and 2 schizoaffective subjects) had significantly elevated levels of HDAC 9 when compared to all other subjects (including controls and patients; $t=2.13$; $df=62$; $p<0.036$) but not when compared only to other patients being treated with psychotropic medications (only patients $p=$ns). All other HDAC enzymes were comparably expressed in the valproic acid treated group. No other systematic analyses were attempted with the other medications given the number of subjects across diagnostic groups with multiple prescribed medications of currently unknown effects on epigenetic enzyme systems.

## 4. Discussion

The availability of a well characterized microarray database allows for the preliminary test of a hypothesis in-silico. Given the validity of the technology, the caveat is to avoid analyzing a broad array of targets thereby creating an over-determined statistic as we have previously reported (Gibbons et al., 2005). We surveyed expression levels in *nine* HDAC genes, and accepted *six* for statistical analysis. This effort is a direct extension of our ongoing examination of chromatin structure in schizophrenia and bipolar subjects (Sharma et al., 2005; Sharma et al., 2006, Gavin et al., 2007).

During the preparation of this manuscript, Benes et al. (2007) reported elevated HDAC1 levels from an independent sample of postmortem hippocampal tissue (seven normal, seven schizophrenia and seven bipolar subjects) using laser microdissected samples of GABA neurons obtained from the stratum oriens of CA2/3 layers. They reported elevated levels of HDAC1 by microarray analysis, which was validated using quantitative RT-PCR. Overall a 2 fold increase of HDAC1 in the GABA neurons of the stratum oriens of the CA2/3 region of the hippocampus from schizophrenia subjects was found.

The increase in HDAC1 in the prefrontal cortices of schizophrenia subjects (as well as the specific region of the hippocampus as described by Benes et al., 2007) is noteworthy because enhanced expression of these enzymes is predictive of chromatin that is deactylated at site specific residues and consequently repressive to transcriptional activity. Akbarian et al. (2005) have examined histone modifications in protein extracts from the prefrontal cortices of schizophrenia patients and normal subjects using Western blot. Although not finding overall differences in levels of modified histones, they report reduced levels of targeted gene expression in subsets of patients with higher levels of

specific modifications including acetylation at H3 Lysine 14. We have earlier reported that histone protein obtained from the peripheral lymphocytes of clinical schizophrenia patients is less acetylated than that obtained from bipolar patients, and remains unyielding when subjects are treated with an HDAC inhibitor (Sharma et al., 2006). Other details of our analysis that should be considered preliminary are gender differences particularly in the schizophrenia subsample, where female schizophrenia subjects ($n=3$) had almost twice the amount of HDAC1 in their prefrontal cortices than did their male counterparts ($n=13$) (516.86 vs. 265.33; $p<0.003$). Also, the confounding effects of psychotropic medication cannot be entirely resolved in this clinical sample because of multiple medications and lengths of treatment. Preliminary observations from our lab indicates that HDAC inhibitors such as Trichostatin A and Valproic acid do not reliably change HDAC1 mRNA expression in either primary lymphocyte cultures from human subjects (Gavin and Sharma unpublished) or in NT2 dividing cells (Kundakovic and Grayson unpublished). However, MS-275, a new investigational HDAC inhibitor, can downregulate protein levels of HDAC1 in NT2 cells (Kundakovic and Grayson personal communication). Furthermore, the effects of the typical and atypical antipsychotics as well as antidepressant medications could not be systematically explored. The effects of valproic acid on HDAC9 are interesting and should be considered preliminary. Nonetheless, the effects of psychotropic medications on chromatin structure and its attendant enzymes will be a fruitful area for future investigation.

Prior basic work in cell lines (Kundakovic et al., 2006), primary neuron cultures (Noh et al., 2005; Sharma unpublished data), rodent models (Tremolizzo et al., 2002, 2005, Simonini et al., 2006) and peripheral blood lymphocytes from human subjects (Gavin et al., 2007) indicate that the GAD67 promoter is regulated by epigenetic mechanisms. These studies show that GAD67 expression is profoundly impacted by the acetylation of histone proteins surrounding its promoter as well as the intensity of DNA methylation in this region. Our observation of a negative correlation between the expression of GAD67 and several HDAC enzymes is encouraging in that it provides independent validity to the relevance of these findings, and is consistent with previous published reports.

HDAC1 is a nuclear protein of the Class I type (de Ruijter et al., 2003). It is ubiquitously distributed (brain as well as lymphoid tissue for example), is activated by phosphorylation and associates with larger multiprotein repressor complexes (such as Co-REST in neurons) to stabilize its enzymatic activity (Saha and Pahan 2006). HDAC1 is also inhibited by small molecule pharmacological agents such as the widely prescribed mood stabilizer valproic acid (mM range) and Trichostatin A (nM range). Deactylase activity occurs in partnership with DNA binding proteins that not only recruit the enzyme to the targeted promoter but also enhance catalytic activity. HDAC1 has two phosphorylation sites ($Serine^{421}$ and $Serine^{423}$) which regulate catalytic activity as well as protein–protein complex formation.

Enhanced deactylase activity and restrictive chromatin in the prefrontal cortices of schizophrenia patients is a heuristic explanation for a variety of clinical findings, from reduced metabolism, cognitive disturbances, negative symptoms and low motivation. Alternatively, restrictive chromatin may be a consequence of the impoverished environmental, emotional and cognitive stimulation that is the daily experience of these subjects or even perhaps the cognitive blunting imposed by ongoing antipsychotic treatment. In either case, a catalytic enzyme serves as an ideal target for pharmacology and a realistic approach to regulate gene expression in a targeted brain region.

**Role of funding source**

RPS is supported by MH 69839, DRG by MH62682, and DPG by MH067631. The NIMH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

**Contributors**

RPS conceptualized the study and analyzed the microarrays. DPG and DRG performed the literature searches, and assisted with the analyses in addition to working on numerous drafts of the manuscript. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

No conflicts of interest declared.

**Acknowledgements**

The authors acknowledge the support and critical suggestions of Dr Francine Benes and the National Brain Databank funded through an NIH grant (R24 MH/NS31862) awarded to the Harvard Brain Tissue Resource Center at McLean Hospital.

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