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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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**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

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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.