Epigenetic modifications in frontal cortex from Alzheimer’s disease and bipolar disorder patients

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Alzheimer’s disease (AD) and bipolar disorder (BD) are progressive brain disorders. Upregulated mRNA and protein levels of neuroinflammatory and arachidonic acid (AA) markers with loss of synaptic markers (synaptophysin and drebrin) have been reported in brain tissue from AD and BD patients. We hypothesized that some of these changes are associated with epigenetic modifications of relevant genes. To test this, we measured gene-specific CpG methylation, global DNA methylation and histone modifications in postmortem frontal cortex from BD (n = 10) and AD (n = 10) patients and respective age-matched controls (10 per group). AD and BD brains showed several epigenetic similarities, including global DNA hypermethylation, and histone H3 phosphorylation. These changes were associated with hypo- and hypermethylation of CpG islands in cyclooxygenase-2 and brain-derived neurotrophic factor promoter regions, respectively. Only the AD brain showed hyper- and hypomethylated CpG islands in promoter regions for cAMP response element-binding protein and nuclear transcription factor kappa B genes, respectively. Only the BD brain demonstrated increased global histone H3 acetylation and hypermethylation of the promoter region for the drebrin-like protein gene. There was no significant epigenetic modification for 12-lipoxygenase or p450 epoxygenase in either illness. Many observed epigenetic changes were inversely related to respective changes in mRNA and protein levels. These epigenetic modifications involving neuroinflammatory, AA cascade and synaptic markers may contribute to progression in AD and BD and identify new targets for drug development.

Introduction

Alzheimer’s disease (AD) and bipolar disorder (BD) are progressive neuropsychiatric illnesses with overlapping symptoms and neuropathology, including brain atrophy, cognitive impairment, emotional disturbances, neuroinflammation, excitotoxicity and upregulated brain arachidonic acid (AA) metabolism.1–3 Common behavioral disturbances in AD, aside from memory loss, are apathy, depression, agitation and general withdrawal. Apathy is the most prevalent disturbance, affecting about 70% of AD patients; depression ranks second, occurring in about 54% of patients; and agitation ranks third, appearing in about 50% of patients.5 Progressive neurostructural changes have been reported in adolescent and adult patients with BD,5,6 associated with cognitive impairment.7 Although genome-wide studies have identified a number of potential risk alleles for BD and late-onset AD, the contribution of each is small and explains only a fraction of the known heritability.8–11 High throughput genetic analysis confirms that neuropsychiatric disorders are very complex and involve many small interdependent genetic abnormalities that are influenced by polygenic inheritance, epigenetic interactions and pleiotropy.12 Several studies have implicated epigenetic mechanisms in these illnesses.12,13 In this study, we examined brain epigenetic changes in AD and BD.

Epigenetic modification in the form of DNA methylation involves covalent addition of a methyl group from the methyl donor S-adenosylmethionine to a cytosine base within the DNA. This reaction is catalyzed by a family of DNA methyltransferases (DNMTs), with DNMT1 and DNMT3A being the main enzymes in mammalian brain.14–16 DNA methylation in the promoter region of a gene has been associated with decreased transcriptional activity.17 CpG islands are extended regions of cytosine and guanine repeats in the promoter region of many mammalian genes. These sites are heavily targeted by DNMTs and are known to modulate gene expression.18

Histones are basic proteins that regulate the compaction of chromatin and can undergo post-translational epigenetic modification by acetylation, methylation, phosphorylation, ubiquitination or sumoylation. Histone acetylation and phosphorylation have been linked to transcriptional activation,19–20 whereas trimethylation of histone-3K4 is suggested to silence gene expression, although the effects of this modification are still under investigation.21–23 Epigenetic studies in neuropsychiatric disorders may identify why behavioral phenotypes among patients are highly variable.16,23

Recently, we reported that AD and BD postmortem frontal cortex (Brodmann area 9) shows upregulation of mRNA and protein levels of neuroinflammatory and arachidonic acid (AA) cascade markers such as AA-selective calcium-independent...
cytosolic phospholipase A2 (cPLA2)-IVA, secretory PLA2 (sPLA2)-IIA and cyclooxygenase-2 (COX-2).\(^2\,^{1,\,26}\) Loss of the synaptic proteins synaptophysin and drebrin is reported in both diseases,\(^2,\,26,\,27\) and excitotoxicity is linked to increased glutamatergic function with loss of excitatory amino-acid transporters in both disorders.\(^2,\,26,\,29\) Brain-derived neurotrophic factor (BDNF) also is reduced in the AD and BD brain.\(^27,\,30\) The increased inflammatory and AA markers in BD are associated with increased mRNA and protein levels of transcriptional factor nuclear factor kappa B (NF-\(\kappa B\)) subunits.\(^2\)

It is not known if the altered mRNA and protein levels of AA cascade, neurotrophic, and synaptic protein markers in AD\(^2\) and BD\(^1\,\,27\) are associated with epigenetic modifications. To test this possibility, in this study we measured global DNA methylation and promoter region-specific CpG methylation for COX-2, 12-lipoxygenase (12-LOX), p450 epoxygenase, BDNF, CREB (cAMP response element-binding), synaptophysin, drebrin-like protein and NF-\(\kappa B\) in the AD and BD frontal cortex tissue in which we had reported changes in mRNA and protein levels of AA cascade, inflammatory and synaptic markers.\(^1\,\,3\,\,3^{\pm}\,2\,7\) We also measured total tissue histone phosphorylation and acetylation. Additionally, we measured mRNA levels of BDNF and NF-\(\kappa B\) subunits in the AD brain samples, as this was not previously determined.\(^2\)

Revealing epigenetic mechanisms that upregulate neuroinflammatory and AA cascade markers and reduce synaptic markers in these disorders could elucidate etiological mechanisms and identify new targets for pharmacotherapy and platforms for diagnosis. We studied frontal cortex because functional and structural abnormalities have been reported in this region in AD\(^3^{\pm}\,36\) and BD patients, and we had studied in previously.\(^3\,\,7\,\,36\,\,37\) An abstract of part of this work has been presented.\(^43\)

Materials and methods

Postmortem brain samples. Frozen postmortem human frontal cortex samples (Brodmann area 9) from 10 AD patients and their 10 age-matched controls, and from 10 BD patients and their 10 age-matched controls, were provided by the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA, USA) under PHS grant number R24MH068855 to JS Rao. The protocol was approved by the Institutional Review Board of McLean Hospital, and by the Office of Human Subjects Research of the NIH (# 4380). Characteristics of the AD, BD and matched control subjects are described in detail elsewhere.\(^2\,\,26\) Briefly, age (years, control: 70.20 ± 2.4 vs AD: 70.60 ± 2.4; control: 43 ± 3.5 vs BD: 49 ± 7.2), postmortem interval (hours, control: 19.16 ± 1.0 vs AD: 19.74 ± 1.0; control: 27 ± 1.5 vs BD: 21 ± 3.0) and brain pH (control: 6.76 ± 0.07 vs AD: 6.84 ± 0.07; control: 6.6 ± 0.16 vs BD: 6.7 ± 0.09) did not differ significantly between the respective groups.

Genomic DNA isolation. Total genomic DNA was isolated from postmortem frontal cortex of AD and BD patients and controls using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich, St Louis, MO, USA). Briefly, tissue was homogenized in lysis solution T and proteinase K solution, and incubated for 4 h at 55 °C in a shaking water bath. Genomic DNA was isolated according to the manufacturer’s instructions.

Gene-specific DNA methylation determination. Gene-specific DNA methylation was determined with a OneStep Methyl-Lite kit (Zymo Research, Irvine, CA, USA) and methyl primer (SABioscience, Frederick, MD, USA), with minor modifications. Promoter methylation for COX-2, BDNF, NF-\(\kappa B\), CREB, 12-LOX, p450 epoxygenase, synaptophysin and drebrin-like genes were studied in AD, BD and control brains. Briefly, 20 ng of global DNA was incubated in the presence (test reaction) or absence (reference reaction) of methyl sensitive restriction enzymes (5 U each) (BSHUI, HpyCH4IV and Hpall, NEB, Ipswich, MA, USA) at 37 °C for 2 h, followed by real-time reverse transcription PCR (RT-PCR) as described in the manufacturer’s instructions. Percentage methylation was calculated using the formula 100 × 2 \(-\Delta C_T\), where \(\Delta C_T\) is the average \(C_T\) value from the test reaction minus the average \(C_T\) value from the reference reaction. Percentage methylation is relative to each experiment.

Total RNA isolation and real-time RT-PCR. Total RNA isolation and RT-PCR was done as described.\(^2\) mRNA levels of BDNF, NF-\(\kappa B\) p50 and NF-\(\kappa B\)p65 were measured by quantitative RT-PCR, using an ABI PRISM 7000 sequence detection system (Applied Biosystems, Carlsbad, CA, USA). Specific primers and probes for BDNF, NF-\(\kappa B\) p50 and NF-\(\kappa B\) p65 were part of the TaqMan gene expression assays (Applied Biosystems), and consisted of a 20 × mix of unlabeled PCR primers and Taqman minor groove binder probe (FAM dye-labeled). The fold-change in gene expression was determined by the \(\Delta \Delta C_T\) method.\(^36\) Data are expressed as the relative level of the target gene in the AD or BD brain normalized to the endogenous control (β-globulin) and relative to the control (calibrator). Experiments were carried out in duplicate.

Global DNA methylation determination. Global DNA methylation was determined from the genomic DNA of AD, BD and matched controls using an Imprint Methylated DNA Quantification Kit (Sigma Aldrich) following the manufacturer’s recommendations. Values are expressed as percent of control.

Global histone, acetylation and phosphorylation determination. Nuclear extracts were prepared from frontal cortices of AD, BD and matched controls as previously described.\(^44\) Global acetylation (H3) and phosphorylation (H3) were measured in the nuclear extracts using ELISA kits (Epigentek Group, Farmingdale, NY, USA).

Statistical analysis. Data are expressed as mean ± s.e.m. T-tests were used to compare AD and BD with matched control group samples.
Results

Hypomethylated COX-2 promoter region in AD and BD. Both AD and BD compared with respective control frontal cortex (Brodmann area 9) show increased AA cascade markers (protein and mRNA levels of cPLA₂-IVA, sPLA₂-IIA and COX-2).¹ ³ There are no recognized CpG islands reported for PLA₂ isoforms. However, the COX-2 promoter CpG region showed decreased methylation in both AD and BD brains (Figures 1a and b). There was no significant change in CpG island methylation for 12-LOX or p450 epoxygenase in either AD or BD (Figures 1c–f).

Hypermethylated BDNF promoter region in AD and BD. There was a significant decrease in BDNF mRNA in the AD brain (Figure 1g). The BDNF mRNA level in BD brain is published elsewhere and also is decreased significantly.² ⁷ Both brains showed a significantly increased methylation state of the promoter region of the BDNF gene (Figures 2a and b). The BDNF transcription factor CREB gene was significantly hypermethylated at its promoter region in the AD but not BD brain (Figures 2c and d).

CpG methylation of synaptic markers in AD and BD. The changes observed in the AD and BD brains are associated with changes in the synaptic proteins, synaptophysin and drebrin.⁵ ¹⁵ There is no recognized CpG island region in the drebrin promoter. Instead, we examined the promoter of drebrin-like protein, which is involved in post-synaptic regulation.⁴⁵ There was a significant increase in DNA methylation at the promoter region of synaptophysin in the AD but not BD brain (Figures 2e and f). The promoter region of drebrin-like protein was significantly hypermethylated in BD but not AD brain (Figures 2g and h).

CpG methylation of NF-κB in AD and BD. BD and AD brains exhibit significantly increased mRNA and protein levels of neuroinflammatory markers such as IL-1β and TNF-α, and of markers of astrocytic and microglial activation.² ³ NF-κB binding sites are present on the promoter region of gene transcripts of AA cascade markers, cPLA₂-IVA, sPLA₂-IIA and COX-2⁴⁶–⁴⁸ and regulate transcription of proinflammatory genes.⁴⁹ ⁵⁰ We tested whether altered expression of these markers was associated with altered methylation states in the NF-κB transcription factor promoter region. The AD brain showed significantly decreased methylation of the NF-κB promoter CpG region, but this was not observed in the BD brain (Figures 3a and b). The hypomethylated state of the NF-κB promoter was accompanied by reciprocal increases in NF-κB p50 and p65 subunit mRNA expression (Figure 3c).

Increased global DNA methylation and altered global histone modification in AD and BD. The AD and BD brains showed significant increases in global DNA methylation compared with respective control levels (Figures 3d and e). These changes were associated with significant increases in H3 phosphorylation in both cases (Figures 3f and g). Global histone H3 acetylation was increased in the BD but not AD brain (Figures 3h and i).

Correlations with brain variables. Pearson correlations between the gene-specific methylation and histone modification levels in AD and BD brains treated separately on the one hand, and postmortem interval, age and pH on the other, were all statistically insignificant (P > 0.05) (Table 2). Mean values of the three parameters did not differ significantly between AD and BD and respective control groups (Table 1).

Discussion

AD and BD are chronic progressive illnesses associated with upregulated mRNA and protein levels of neuroinflammatory markers (GFAP, CD11b, IL-1β) and of brain AA cascade enzymes (cPLA₂-IVA, sPLA₂-IIA and COX-2), as well as loss of neurotrophic factors (BDNF) and presynaptic and postsynaptic proteins (synaptophysin and drebrin).¹ ² ²⁶ ²⁷ Some of these alterations could be related to epigenetic modifica-

Figure 1 Mean levels of CpG methylation at promoter region of cyclooxygenase-2 (COX-2), 12-lipoxygenase (12-LOX) and p450 epoxygenase in frontal cortex of Alzheimer’s disease (AD) (a, c, e) and bipolar disorder (BD) patients (b, d, f). Mean levels of brain-derived neurotrophic factor (BDNF) mRNA in AD and control brain samples (g). Mean ± s.e.m. (n = 10 per group). * P < 0.05, ** P < 0.01, *** P < 0.001.
The current study revealed that the increased COX-2 expression previously reported in the same AD and BD frontal cortex samples may partly be due to the hypomethylated state of the COX-2 CpG promoter region. 26 However, other AA cascade markers, including 12-LOX and p450 epoxygenase, did not have DNA promoter methylation changes. Thus, the changes reported for 12-LOX and p450 epoxygenase mRNA and protein levels 26 are unrelated to methylation at their gene promoter region. Protein and mRNA levels of cPLA2-IVA and sPLA2-IIA are upregulated in both disorders. 1,26 Human promoter regions of cPLA2 and sPLA2 isoforms and neuroinflammatory markers are reported to lack CpG islands (http://genome.ucsc.edu/), so we did not test their methylation states. The upregulation of cPLA2-IVA and sPLA2-IIA...
In conclusion, both the BD and AD frontal cortex exhibits altered epigenetic regulation related to neuroinflammation,
synaptic integrity, neuroprotection and AA metabolism (Table 2). These changes may modify disease progression and could help in identifying new therapeutic routes for treatment and diagnosis.

Conflict of interest

The authors declare no conflict of interest.

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