



Diagnostic Assessment & Prognosis

Developmental lead exposure and lifespan alterations in epigenetic regulators and their correspondence to biomarkers of Alzheimer's disease

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Abstract

Introduction: Early life lead (Pb) exposure results in a latent increase in Alzheimer's disease (AD)-related proteins, and cognitive deficits late in life in both rodents and primates. This study was conducted to investigate if these late life changes were accompanied by epigenetic alterations.

Methods: Western blot analysis and RT-PCR were used to measure Deoxyribonucleic acid methylation regulators (DNMT1, DNMT3a, MeCP2, MAT2A) and histone proteins (H3K9Ac, H3K4me2, H3K27me3).

Results: Cerebral levels of DNMT1 and MeCP2 were significantly reduced in mice exposed to Pb early in life, whereas the expression of DNMT3a was not altered. Levels of MAT2a were increased in the Pb-exposed mice across the lifespan. H3K9Ac and H3K4me2, involved in gene activation, were decreased, whereas the repressive mark H3K27me3 was elevated.

Discussion: Epigenetic modifiers are affected by the developmental exposure to Pb and may play a role in mediating the latent increases in AD-related proteins in the brain.

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Keywords: Aging; Alzheimer's disease; Epigenetics; Lead (Pb); Lifespan

1. Introduction

According to the 2015 Alzheimer's Report, there are currently 5.3 million Americans living with AD [1]. The vast majority of these individuals (>95%) are aged ≥65 years, with a disease subtype known as late-onset AD (LOAD). The remaining 5% of cases make up early-onset AD (EOAD), and of these (<1%) are linked to mutations in one of three genes, *Amyloid Precursor Protein (APP)*, *Presenilin-1 (PSEN1)*, and *Presenilin-2 (PSEN2)* [2]. LOAD is challenging to researchers, the presentation of the disease is sporadic as patients exhibit

non-Mendelian characteristics. To date, there is no clear genetic etiology for LOAD, other than a risk factor of carrying specific susceptibility alleles [3,4]. The single and only proven risk factor for the development of this sporadic form of AD is aging. These observations suggest the involvement of environmental and/or epigenetic factors across the lifespan in the initiation and development of the disease [5].

Early studies have provided evidence that many chronic adult diseases and disorders, such as cardiovascular disease, diabetes, and obesity, are linked to environmental exposures that occurred during development [6,7]. Importantly, there is also a growing body of literature to support the contention that exposures to environmental toxins in early life contribute to the development of at least some of the neurodegenerative disorders [8].

There are few epidemiologic studies examining the link between environmental exposures and development of

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LOAD. These studies have assessed relationships between past exposures to pesticides, metals, dietary changes, and other environmental influences; however, the results have been limited. We have taken a specific interest in the environmental agent and heavy metal lead (Pb). Pb has been implicated as a neurotoxin for generations, and is known to have devastating consequences for the nervous system specifically in children [9]. The metal is nondegradable, ubiquitously found in nature, has high bioavailability, can cross the blood brain barrier and has a long half life [10]. Epidemiologic studies assessing both occupational exposure and exposures in community-dwelling individuals have identified Pb as a clear disrupter of cognitive performance and function [11,12].

Our laboratory was among the first to provide evidence that exposure to an environmental Pb in early life results in latent overexpression of AD-related proteins and histopathology in late life [13–16]. Reports by us have also demonstrated a latent increase in both amyloidogenic and tauogenic proteins, as well as behavioral deficits in our rodent models [13,17]. These results have been replicated and further investigated in a primate model, where we have also reported the presence of tangles and plaques in the cerebral cortex [14,16]. More recently, we have shown that the changes in the expression of proteins such as APP, amyloid beta (A β), and microtubule-associated protein tau are accompanied by epigenetic changes [14,18]. Epigenetics refers to the process that results in modifications in gene activity independent of the primary deoxyribonucleic acid (DNA) sequence [19]. DNA methylation alterations and histone tail modifications are the most widely studied forms of epigenetic modifications.

This study documents the expression of four enzymes important in regulating DNA methylation and three histone modifications (two activating, one repressive) across the lifespan of animals that were exposed to Pb during a brief period of early postnatal life. Our results provide a valuable framework for understanding a number of epigenetic interactions that likely play a critical role in the expression of neurotoxic AD-related proteins in later life. This work is the first to identify epigenetic changes across the lifespan after a developmental exposure that has been previously shown to have significant increases in AD-related biomarkers.

2. Methods

2.1. Animal exposure

C57BL/6 mice were bred in house in the Animal Care Facility at the University of Rhode Island, according to previously published protocols [15,17]. All experiments were performed in accordance with the standard guidelines and protocol approved by the University of Rhode Island Institutional Animal Care and Use Committee (IACUC) with supervision of the university's veterinarian. Males were divided into two groups and used in this study. The

control group received regular tap water, whereas the second group (PbE, developmental exposure) was exposed to 0.2% Pb-acetate from PND 1 to PND 20 through the drinking water of the dam. Animals receiving Pb acetate had no observable adverse developmental deficits. Brains were dissected at the following ages: PND 20, 180, 270, 540, and 700 and stored at -80°C until use. These animals are from the subset of the same cohort that has already been characterized for Alzheimer's pathology and behavioral deficits [15,17].

2.2. Total RNA isolation, synthesis of cDNA, and real-time PCR

Total RNA was isolated from the cerebral cortex after the TRIzol method (Invitrogen, Carlsbad, CA). Samples were checked for integrity and purity by NanoDrop (Thermo Fischer Scientific, Waltham, MA). First-strand complementary DNA (cDNA) was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Approximately 1 μg of RNA was diluted in 19 μL of water, with 4 μL 5 \times iScript reaction mix, and 1 μL of iScript reverse transcriptase. Samples were incubated at 42°C for 90 minutes, followed by 85°C for 5 minutes using the MJ Research MiniCycler PTC-150 (Bio-Rad). Real-time PCR was carried out in 12.5- μL reaction volumes containing 1 μL of cDNA template, 4.75- μL nuclease free water, and 6.25- μL SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Real-time PCR was performed using the ViiA7 Real-Time PCR System (Applied Biosystems) using the following conditions: 50°C for 2 minutes followed by 95°C for 10 minutes then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Expression data were reported using the $2^{-\Delta\Delta\text{CT}}$ method and GAPDH mRNA as the endogenous control. The primer pairs used are summarized in Table 1.

2.3. Protein extraction

For DNMT1, DNMT3a, and MECP2, the nuclear fraction was collected according to the method described by Dignam et al, with minor modifications [20]. Samples were homogenized in 1-ml phosphate buffer saline at pH 7.4 followed by centrifugation at $2500 \times g$ for 10 minutes. The pellets obtained were resuspended in 5 volumes of buffer A (10-mM HEPES, pH 7.9, 1.5-mM MgCl_2 , 0.5-mM DTT, 0.5-mM EDTA, and 0.2-mM PMSF) and centrifuged at $600 \times g$ for 2 minutes at 4°C . The pellets were resuspended in 3 volumes of buffer A and centrifuged at $600 \times g$ for 2 minutes at 4°C . After centrifugation, pellets were then resuspended and homogenized in 5 vol of buffer C (20-mM HEPES, pH 7.9, 1.5-mM MgCl_2 , 0.5-mM DTT, 0.5-mM EDTA, 420-mM NaCl, 20% glycerol, 0.2-mM PMSF, 2- $\mu\text{g}/\text{ml}$ aprotinin, and 0.5- $\mu\text{g}/\text{ml}$ leupeptin). Suspensions were centrifuged at $12,000 \times g$ for 10 minutes at 4°C . supernatants were collected, frozen, and stored at -80°C until use. Cytoplasmic protein samples were isolated using the NE-PER

Table 1
Primer sequence pairs used in the study for the analysis of gene expression

Gene	Forward primer	Reverse primer
<i>DNMT1</i>	5'-GAGTCTTCGACGTCACACCA-3'	5'-AGCTACCTGCTCTGGCTCTG-3'
<i>DNMT3a</i>	5'-CTGGAGAAGCGGAGTGAAC-3'	5'-GGATTGATGTTGGTCTGT-3'
<i>MeCP2</i>	5'-CAGCAGCATCTGCAAAAGAAG-3'	5'-TCCACAGGCTCCTCTCTGTT-3'
<i>MAT2A</i>	5'-ACCCTATGCATGGTTTCAGC-3'	5'-ACCCTGGGAGGAGCTATTG-3'
<i>GAPDH</i>	5'-AGGTCGGTGTGAACGATTG-3'	5'-TGTAGACCATGTAGTTGAGGTCA-3'
<i>B-ACTIN</i>	5'-TGTTACCAACTGGGACGACA-3'	5'-TCTCAGCTGTGGTGGTGAAG-3'

nuclear and cytoplasmic extraction reagents according to the manufacturer's instructions (Thermo Fischer Scientific).

2.4. Histone extraction

Total histones were extracted using the EpiQuick Total Histone Extraction Kit (Epigentek, Farmingdale, NY) following the manufacturer's instructions, with minor modifications. Cortical tissue was weighed and cut into 1–2 mm pieces and homogenized at 200 mg/mL with prelysis buffer. The homogenate was centrifuged at 10,000 rpm for 1 minute at 4°C. The supernatant was discarded, and the pellet was re-suspended in 3 × volume of lysis buffer and incubated on ice for 30 minutes. The sample was centrifuged at 12,000 × g for 5 minutes at 4°C, and the supernatant was transferred to a fresh vial. Balance buffer was added to the supernatant (0.3 mL to 1 mL supernatant). The extract was placed at –80°C for long-term storage.

2.5. Western blot analysis

Protein concentration was determined by using the Micro BCA Protein Assay Kit (Thermo Fischer Scientific). Protein samples were then denatured and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 150 V for 1–2 hours and then transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Piscataway, NJ), blocked for 1 hour with 5% bovine serum albumin and then immunoblotted with the appropriate primary antibody. For MAT2A, 40 µg of cytoplasmic extracts were separated on 10% SDS-PAGE gels and incubated overnight with primary polyclonal antibody at the dilution 1:1000 of ab77471 (Abcam, Cambridge, UK). Membranes were then stripped and reprobed with 1:2000 dilution of monoclonal A3854 (Sigma-Aldrich, St. Louis, MO) for β-actin. For DNMTs and MeCP2 proteins, 100 µg of nuclear extracts were separated on 5% SDS-PAGE gels and incubated overnight with primary monoclonal antibodies (Cell Signaling, Danvers, MA) 1:1000 dilution of D59A4 for DNMT1, D23G1 for DNMT3a, and D4F3 for MeCP2. Membranes were then stripped and reprobed with 1:2000 dilution of G8795 (Sigma-Aldrich, St. Louis, MO) for Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). For histone proteins, the following primary polyclonal antibodies (Epigentek) were used at a 1:5000 dilution, A-4022 for H3K4me2, A-4022 for

H3K9Ac, A-4039 for H3K27me3, A-4035 for H3K9me3, and A-1112 for total H3. Ten micrograms of histones were separated onto 16% gels and incubated overnight with the respective primary antibody. Membranes were then washed four times with tris buffered saline with Tween 20 (TBST) and incubated with appropriate infrared-labeled secondary antibody (Li-Cor, Lincoln, NE) at 1:10,000 for 1 hour at room temperature in the dark. Infrared band signals were detected and quantified using an Odyssey Infrared Imaging System (Li-Cor). MAT2A protein was normalized against β-actin, whereas nuclear proteins were normalized against levels of the housekeeping protein GAPDH. Histone proteins were normalized against bands for total H3.

2.6. Statistical analysis and data representation

Western blot analysis was performed using the Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE). Real-time PCR analysis was completed using the applied biosystems ViiA7 software. Each data set has either an n = 3 or n = 4 (designated in the figure legend), the animals were measured independently without pooling of brain tissue or samples. Individual data points are presented on each graph, as well as the mean for each group. The vertical bars represent 95% confidence intervals and are shown for each data set. The significance of difference between different treatment groups was determined by one-way analysis of variance (ANOVA), with the Holmes–Sidak post hoc test using IBM SPSS statistics 21 software. The significance of interaction between treatment groups and time was determined by two-way analysis of variance. The level of significance was set at α = 0.05. The change in protein levels with respect to each time point was analyzed by ANOVA with the Dunnett's post hoc test.

3. Results

We have previously measured Pb concentrations in animals exposed under the same protocol as the one in this manuscript and found the concentration of Pb in the cerebellum of PND 20 rodents (0.25 ± 0.07 µg/g) to be approximately three times the level seen in control animals [21]. Blood levels have been shown to be 46.43 µg/dl during Pb exposure but are reduced to basal levels in adults [13]. The current recommended levels by the CDC is that of

5 µg/dL, although the blood levels in our exposure scenario are higher, they are still consistent with the levels seen in both children and adults exposed to environmental Pb [22,23].

3.1. Effect of Pb exposure on the expression of DNMT1 and 3a protein

There was no significant interaction effect between Pb treatment and time. The exposed mice had a statistically significant decrease in normalized DNMT1 levels at all timepoints across lifespan relative to the control animals. In both the control group and Pb-exposed animals, DNMT1 protein expression was decreased at all timepoints relative to PND 20 (Fig. 1A). Unlike DNMT1 protein levels, DNMT3a showed no significant difference between control and Pb-exposed animals across the lifespan of the mice (Fig. 1B).

3.2. Levels of MeCP2 protein across the lifespan after exposure

MeCP2 protein levels normalized relative to GAPDH are increased at day 270 in both the control and Pb-exposed animals; however, these results are not significant. Statistically significant decreases between the Pb-exposed group and the control group are observed at day 180 and 540 as seen in Fig. 2A. The remaining timepoints exhibited no significant difference between Pb exposed and controls. The control group exhibited no differences in proteins levels from PND 20 at any other time point. Whereas in the Pb-

exposed animals, there was significant differences in protein expression observed at all timepoints except for PND 270. There was also no significant interaction effect between Pb treatment and time.

3.3. Protein levels of MAT2A protein across the lifespan and after Pb exposure

The cytoplasmic protein expression profile of MAT2A across the lifespan of control groups did not display any significant change (Fig. 2). However, mice exposed to Pb as infants demonstrated a statistically significant increase in levels of MAT2A at day 270, 540, and 700 relative to control. Similarly, there is no interaction effect between time and treatment.

3.4. Alterations in gene expression of DNA mediators after Pb exposure

Changes in gene expression of *Dnmt1*, *Dnmt3a*, and *Mecp2* were also examined by real-time PCR analysis. We observed no significant difference between mRNA levels of *Dnmt1*, across the lifespan between the control and Pb exposed animals (Fig. 3A). *Dnmt3a* mRNA levels for the Pb-exposed animals were elevated compared to controls across the lifespan and significantly increased at PND 20 (Fig. 3B). In Fig. 4C, mRNA for *MeCP2* is significantly lower at PND 20 and PND 270 for the exposed group, with no change at any other time point. There was no significant interaction effect for any of these mRNA targets.

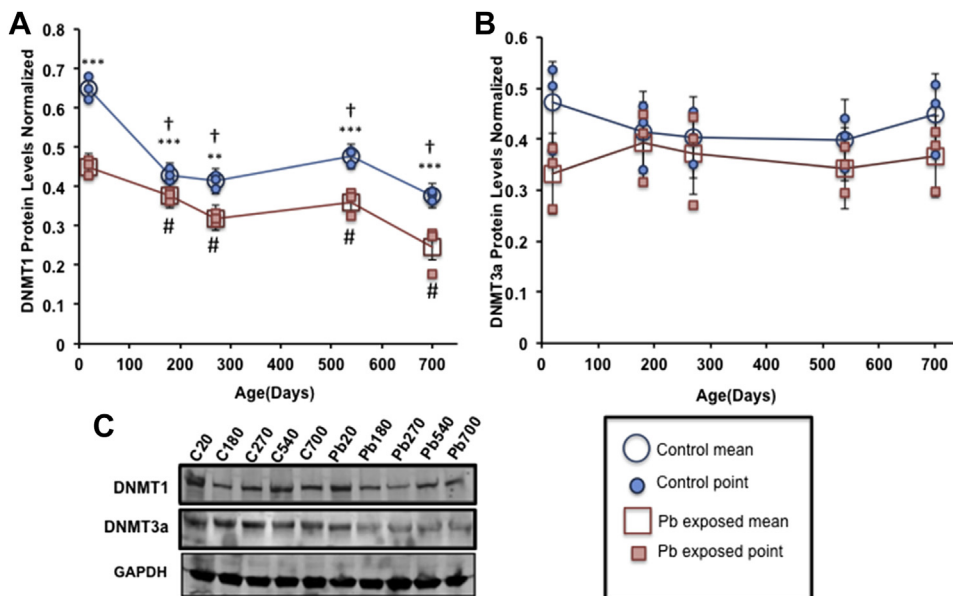


Fig. 1. DNA methyltransferase (DNMT) protein levels across the lifespan after developmental exposure to lead (Pb). (A) Quantification of DNMT1 protein levels across the lifespan for control animals and those developmentally exposed to Pb. (B) Quantification of DNMT3a protein levels across the lifespan after developmental Pb exposure. (C) Representative DNMT1 and DNMT3a proteins levels. Nuclear extracts were used, and proteins were measured by western blot analysis as described in the methods section. Individual data points are represented on the graph, as well as the means for each time point. N = 3 and significance is represented as ** $P < .01$, *** $P < .001$. †Significance from the PND20 group for the control animals, while # is used to denote significance from the PND20 time point for the lead exposure group.

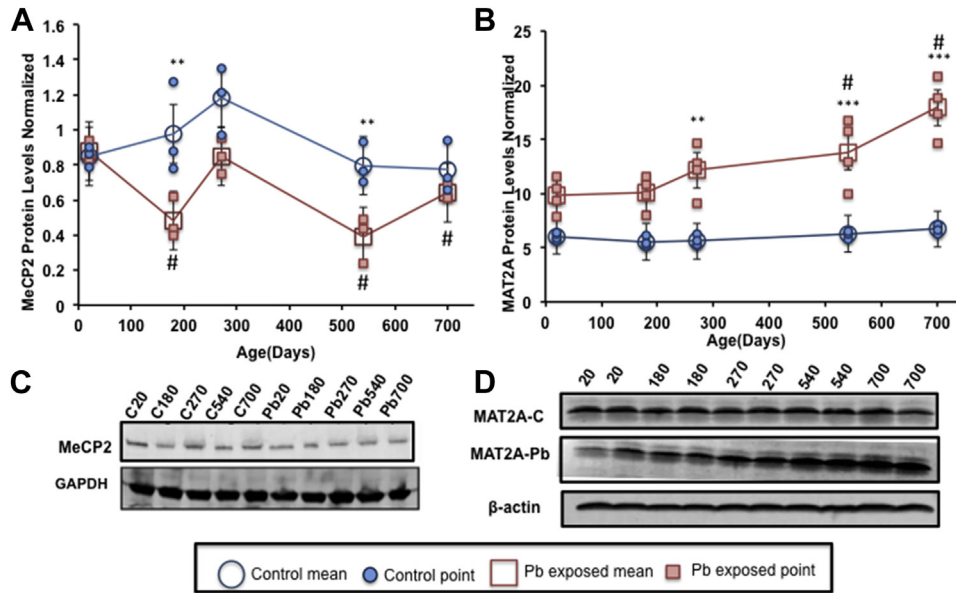


Fig. 2. Methyl CpG binding protein 2 (MeCP2) and Methionine adenosyltransferase 2a (MAT2a) levels across the lifespan after developmental exposure to lead (Pb). (A) Quantification of MeCP2 protein expression levels across the lifespan for control animals and those developmentally exposed to Pb. (B) Quantification of MAT2A protein expression levels across the lifespan for control animals and those developmentally exposed to Pb. (C and D) Representative MeCP2 and MAT2A protein levels. Nuclear and cytoplasmic extracts were used, and proteins were measured by western blot analysis as described in the methods section. Individual data points are represented on the graph, as well as the means for each time point. $N = 3$ for MeCP2 and $N = 4$ for MAT2A, and significance is represented as $**P < .01$, $***P < .001$. #Significance from the PND20 time point for the lead exposure group.

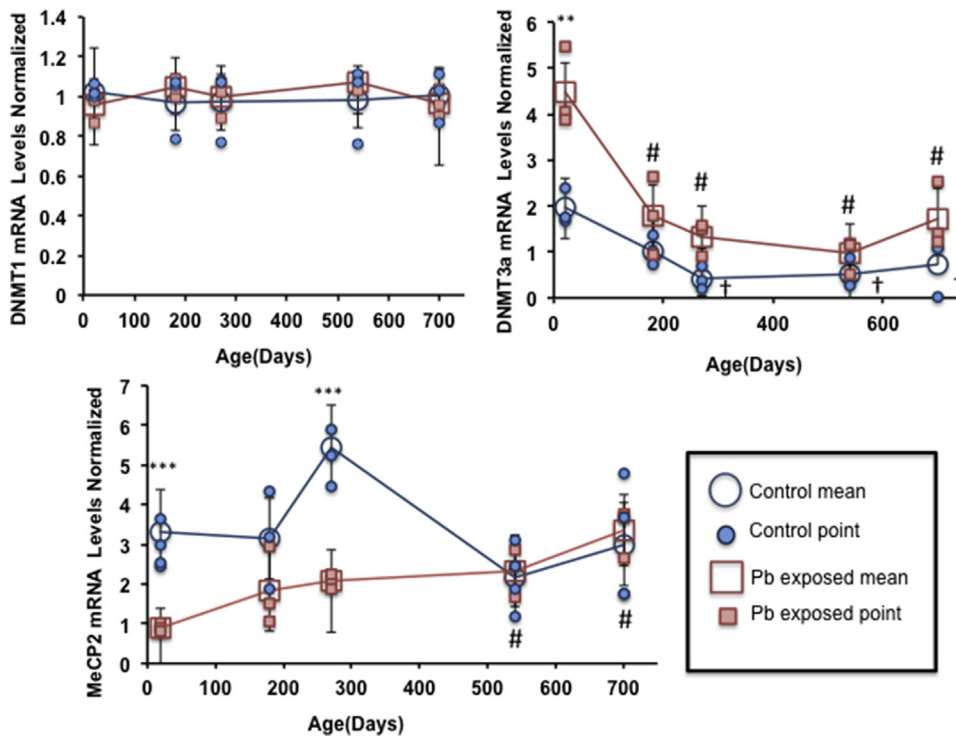


Fig. 3. mRNA levels of DNA methylation regulators across the lifespan after developmental exposure to lead (Pb). (A) Quantification of *Dnmt1* mRNA across the lifespan for control animals and those developmentally exposed to Pb. (B) Quantification of *Dnmt3a* mRNA across the lifespan for control animals and those after infantile exposure. (C) Quantification of *Mecp2* mRNA across the lifespan for control animals and those developmentally exposed to Pb. Individual data points are represented on the graph, as well as the means for each time point. $N = 3$ or $N = 4$ and significance is represented as $**P < .01$, $***P < .001$. † Significance from the PND20 group for the control animals. # Significance from the PND20 time point for the lead exposure group.

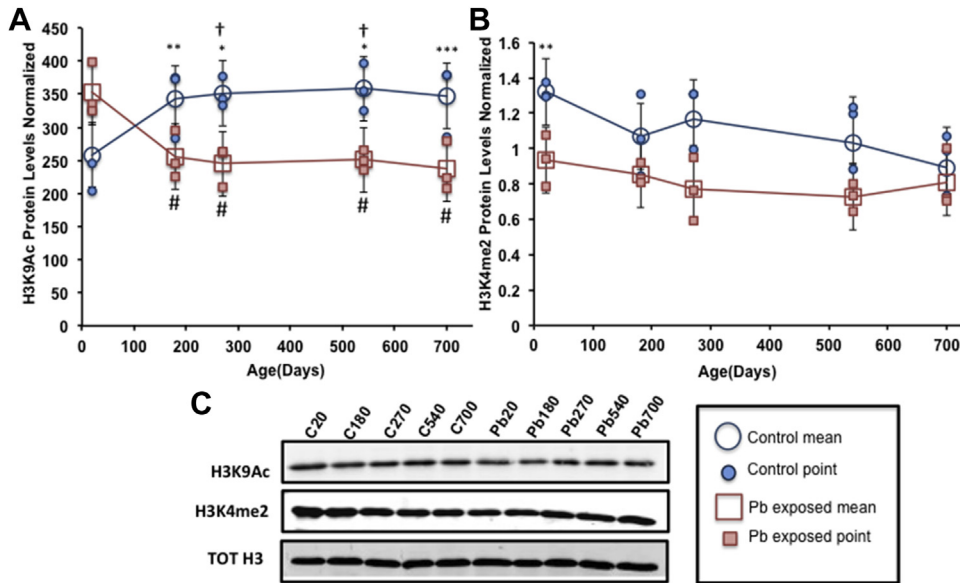


Fig. 4. Activating histone protein levels across the lifespan after developmental exposure to lead (Pb). (A) Quantification of H3K9Ac protein expression levels across the lifespan for control animals and those developmentally exposed to Pb. (B) Quantification of H3K4me2 protein levels across the lifespan after developmental Pb exposure. (C) Representative histone proteins levels. Histone extracts were used, and proteins were measured by western blot analysis as described in the methods section. Individual data points are represented on the graph as well as the means for each time point. Individual data points are represented on the graph, as well as the means for each time point. $N = 3$ and significance is represented as $*P < .05$, $**P < .01$, $***P < .001$. †Significance from the PND20 group for the control animals. #Significance from the PND20 time point for the lead exposure group.

3.5. Effect of Pb on activating and repressive histone marks across the lifespan

Histone extracts were used to profile histone modification marks across the lifespan via western blot. The activating marks are shown in Fig. 4. H3K9Ac protein levels were significantly lower in the Pb-exposed mice for all timepoints except PND 20, as seen in Fig. 4A. In the control animals, there were significant differences compared to PND 20 at both PND 270 and 540, and in the Pb-exposed animals, significance was observed at all timepoints relative to day 20. H3K4me2 levels have a similar trend, the Pb exposed mice have lower protein levels compared to controls across the lifespan, with the only significant decrease occurring at PND 20 (Fig. 5B). Similarly, there were no significant changes at any of the timepoints relative to day 20 in either the control group or Pb-exposed group. Representative blots for H3K9Ac and H3K4me2 are seen in Fig. 4C, with total Histone H3 as a loading control. Protein levels for H3K27me3 are shown in Fig. 5. H3K27me3 is a histone mark indicative of gene repression. Pb-exposed animals showed significantly higher levels of H3K27me3 across the lifespan except for day 180. In the control animals, all timepoints exhibited significant differences relative to PND 20, whereas in the Pb-exposed animals significance was only observed at PND 270 and PND 540. A representative blot for H3K27me3 is shown in Fig. 5B.

4. Discussion

Epigenetic regulation is a complicated phenomenon with converging pathways involved in the regulation of gene

expression. DNA methylation is maintained by DNA methyltransferases such as DNMT1, DNMT3a, and DNMT3b and involves the recruitment of MeCP2 and other proteins [24]. Typically, recruitment of methyl groups and methyl binding proteins is indicative of a decrease in gene expression and is associated with repressive complexes [25].

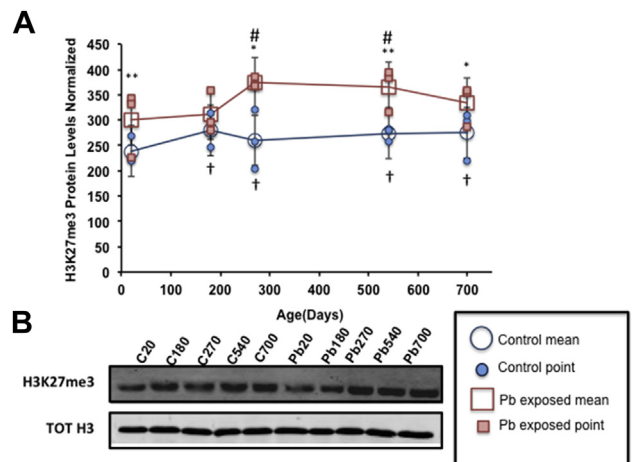


Fig. 5. Repressive histone mark levels across the lifespan after developmental exposure to lead (Pb). (A) Quantification of H3K27me3 protein expression levels across the lifespan for control animals and those developmentally exposed to Pb. (B) Representative H3K27me3 protein levels. Histone extracts were used, and proteins were measured by western blot analysis as described in the methods section. Individual data points are represented on the graph, as well as the means for each time point. $N = 3$ and significance is represented as $*P < .05$, $**P < .01$. †Significance from the PND20 group for the control animals. #Significance from the PND20 time point for the lead exposure group.

Histone tail acetylation is associated with regions of chromatin that are open to transcription and typically indicate gene activation. Histone methylation marks such as H3K27me3 and H3K9me3 are associated with regions of condensed chromatin and gene repression.

This in-vivo study uncovered a significant decrease in DNMT1 levels relative to their age-matched controls across the lifespan of mice developmentally exposed to Pb, with a significant decrease in MeCP2 levels at some timepoints as well. These findings are consistent with our previously published results indicating that differentiated SHSY5Y cells treated with increasing concentrations of Pb have a significant decrease in DNA modification enzymes and MeCP2 [18]. DNMT1 is an important regulator of tissue-specific DNA methylation patterns; exposure to metals such as Cd has been shown to alter the activity and protein levels of this enzyme [26]. We have also previously examined the ability of Pb to alter the activity of DNMT1 in tissue and have found that exposure to Pb does decrease DNMT1 activity [14]. Studies from other groups have also examined changes in protein levels of DNMT1 and MECP2 as a result of postnatal and perinatal Pb exposure [27]. They reported a downregulation in these DNA methylation modifiers in the hippocampus at PND 55 after Pb exposure. Our results are consistent with their findings, indicating that Pb exposure down-regulates these proteins in the cerebral cortex, and that this repression is never recovered. The data points collected in our study indicate that even at PND 700 there is downregulation of DNMT1 and MeCP2 is maintained.

The results obtained from studying the gene expression of these DNA methylation regulators leads us to believe that Pb is most likely decreasing their protein expression directly and perhaps not through transcriptional regulation. There was no significant change in mRNA levels for DNMT1, but there was a decrease in protein relative to controls across the lifespan. A possible mechanism for this may be due to increases in protein degradation of DNMT1 after exposure. Similarly, MeCP2 mRNA was only significantly lower at the PND 20 and PND 270 time point. The most interesting observation obtained from the gene expression data is for DNMT3a. DNMT3a gene expression for the Pb treatment was higher across the lifespan and significantly increased at PND 20, whereas the protein levels were not significantly different in the lead exposed animals relative to control. These data are explained by a recent finding from our laboratory demonstrating increase in miR-29b (targeting DNMT3a) following exposure at PND 20, suggesting that miR-29b may be inhibiting the translation of the DNMT3a mRNA [28]. The same article reported an increase of miR-106 (targeting APP) at PND 700 [28]. There is also evidence that miRNA expression is effected by chronic Pb exposure in an adult rat hippocampus [29]. Our observations with DNMT3a are also consistent with previously published work by our laboratory indicating that DNMT3a gene

expression is 2-fold decreased at PND 700 for the Pb-treated group as compared to PND 20 in primates [30].

MAT2A catalyzes the formation of S-adenosylmethione (SAM) from methione and ATP and is an important regulator of the methylation cycle. It has been well documented that SAM levels have been found to be significantly decreased in the cerebrospinal fluid of patients with AD [31]. In our study, we have shown that Pb-exposed mice have elevated levels of MAT2A across the lifespan compared to their age-matched controls. A recent study in hepatoma cells has shown that on hypoxia exposure, DNA demethylation pathways are activated, whereas MAT2A expression is upregulated [32]. To better understand the role of MAT2A and the changes of gene expression, we will have to further investigate its role in regulating the DNA methylation pathway in our model.

Histone modifications refer to additions of chemical groups to N-terminus tail of histone proteins, most commonly the histone core proteins, H3 and H4. Histone acetylation levels are governed by histone acetyltransferases (HATs) and deacetylases (HDACs) whereas histone methylation by histone methyltransferases (HMTs). It is known that histone modifications and DNA methylation interact to alter the conformation and thus the accessibility of the promoter and coding regions of the genome [33]. In this study, we have shown that early life Pb exposure was found to decrease H3K9Ac and H3K4me2 proteins levels, both of which are marks for gene activation. We have also shown that H3K27me3 levels are increased, which also is indicative of gene repression. These are consistent with our previous findings in our primate tissue, where we observed that histone repressive marks were upregulated, and most genes are downregulated after Pb exposure [34].

Although much information can be gained from understanding the changes in the regulation of DNA methylation enzymes and proteins, we are limited in that we have not identified specific genes that are directly affected in the alteration of binding of these modifiers. We are also trying to investigate the mechanisms by which Pb exposure may contribute to the dysregulation of these proteins. It appears from these studies and previous studies, that after Pb exposure, there are groups of genes that are upregulated, and others that are repressed. This upregulation of specific genes may be facilitated by DNA hypomethylation, whereas the gene repression may be histone mediated. The changes that have been previously reported in this animal model report increases in AD-related proteins late in life, whereas the dysregulation observed in the epigenetic regulators occurs throughout the lifespan [15,17]. Future studies will examine the alterations in DNA methylation, as well as histone modifications that occur at specific genes and the consequences that have the expression of those genes. The implications of this work and the findings obtained from studying histone modifications and DNA methylation are vast. Targeting epigenetic marks is becoming recognized as a novel therapeutic approach for neurodegenerative

disease, including AD [35]. Treatments that have been explored include histone deacetylase (HDAC) inhibitors, such as valproic acid and S-adenosylmethionine supplementation as a method to target DNA methylation changes [35].

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RESEARCH IN CONTEXT

1. Systemic review: We have performed a thorough literature review to understand the scope of environmental risk factors associated with Alzheimer's disease (AD), and the potential mechanisms that mediate their action at various stages of life. We have identified and cited properly past findings on the environmental exposures that impact AD-related genes and the epigenetic mediators associated with them.
2. Interpretation: Our findings connect past findings reported to occur to AD-related genes to the epigenetic mechanisms that regulate them. Our results show a link between early life exposure to Pb and its ability to reprogram the expression of epigenetic intermediates involved in DNA methylation or histone modification that in turn regulate latent AD-related gene expression.
3. Future directions: Our findings have helped us understand that DNA methylation and histone modifications may partially explain the reprogramming of gene expression in old age due to developmental exposure. In the future, we plan to study the role of microRNAs in this process.

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