The role of epigenetics in the treatment of Alzheimer's disease

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THE ROLE OF EPIGENETICS IN THE TREATMENT
OF ALZHEIMER’S DISEASE

by

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VISHNUKARTIK NITTA

ABSTRACT

Epigenetic mechanisms play tremendous roles in the development and management of neural processing. The important mechanisms include inactivation of transcription via methylation, histone modification via acetylation/deacetylation, and miRNA regulation. These modifications allow for expression or silencing of genes, without manipulation of nucleotide sequence. An individual’s internal and external environments provide input for quotidian epigenetic regulation. Aberrations in the form of regulation have been increasingly linked to neurological disorders, in addition to the established correlation to tumorigenesis. In recent years, deviations from normal epigenetic patterns have been observed in cases of Alzheimer’s disease (AD). The brains of patients with AD have been shown to display significantly less methylation overall, as compared to age-matched controls. Of particular concern, the methylation, which normally keeps the promoter of the APP gene silenced, occur far less frequently in AD patient allowing for the progression of amyloid deposition and subsequent tau pathology. In addition to the hypomethylation present in AD, many AD cases present with a concurrent hypoacetylation on histones in the hippocampus. There is strong evidence suggesting that the reduced levels of acetylation are due to over-activation of histone deacetylases. Post-mortem examinations of the brains of AD patients have shown that the
brain-derived neurotrophic gene, which is crucial for neural processing associated with maturation and memory, has low levels of acetylation halting its transcription. While low levels of methylation and acetylation seem to contribute to the pathogenesis of AD, regulatory miRNA levels can have adverse effects whether they are aberrantly reduced or increased. Patients with AD tend to show abnormally augmented expression of miRNA-125b, miRNA-128, and miRNA-9 in the hippocampus, while a reduced expression of miRNA-107. Deregulation of these miRNAs have been linked to the progression of AD and include amyloid deposition, tau pathology, and oxidative stress through inflammatory processes. The latter quandary of oxidative stress has been shown to be crucial for the early progression of AD. Reactive oxygen species disallow the methylation of genes due to steric hindrance at the CpG islands of DNA where DNA methyltransferases act. Research shows that increases in oxidative stress are correlated to decreases in methylation, which allows for APP expression. While these alterations to normal epigenetic patterns occur internally, there is a breadth of changes that the external environment imposes to exacerbated AD pathogenesis. Most heavily studied of these external environmental factors is lead exposure. There is a strong correlation between lead exposure in individuals who carry the ApoE4 gene and increased mRNA transcription of the APP gene. Lead is thought to demethylate the promoter of the APP gene and allow for amyloid processes to occur. Inadequate nutrition, specifically deficits in choline and folate, has been linked to hypomethylated states due to an inefficient “methylation/remethylation cycle” leading to an accumulation of homocysteine characteristic of AD.
With the emphasis epigenetic deregulation has in the progression of AD, epigenetic treatments need to be seriously considered as therapeutic avenues. Current drugs treat the symptoms and acute conditions of AD, but through epigenetic modifications, the pathology of the diseases can be directly addressed. Potential therapeutic avenues include the use of methyl donors, highly specific histone deacetylase inhibitors, and miRNA biomarkers. Methyl donors can help alleviate the hypomethylated state and prevent further APP expression and amyloid deposition. Currently, the histone deacetylase inhibitors are being used as global inhibitors, but have adverse effects including non-specific and premature cell death. By further researching these inhibitors and finding a mechanism to attack specific histone deacetylases (such as HDAC6 in AD), the efficacy of this aspect of treatment will be greatly increased.

The current use of miRNAs as epigenetic regulators to turn off unwanted genetic expression is ineffective due to a major problem of effective delivery to target zones. By using the gene sequences of miRNAs as biomarkers, an AD patient’s genomic sequence can be mapped, marking which areas require regulation. This process is necessary because of the inter-individuality of miRNA regulation between each case of AD. Also, the problem of some anti-miRNA molecules not being able to cross the blood brain barrier needs to be addressed using a novel transport mechanism, as direct brain injections are not feasible. The simplest, and highly effective, therapeutic avenue is a healthy lifestyle. Daily exercise and proper nutrition hinder inflammatory process and oxidative stress and can prevent progression of AD through allowing higher brain perfusion for cognitive functioning.
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LIST OF ABBREVIATIONS

5-Mc: 5-Methylcytosine
8-OHG: 8-hydroxyl-2’-deoxyguanos
Aβ: Amyloid beta
ACE: Angiotension Converting Enzyme
Ach: Acetylcholine
AD: Alzheimer’s Disease
ApoE: Apolipoprotein E (gene)
APOE: Apolipoprotein E (protein)
APP: Amyloid Precursor Protein
BACE1: beta-site amyloid precursor protein cleaving enzyme 1
DNMT: DNA methyl transferases
DUSP22: Dual-Specific Phosphatase-22
ERK: extracellular signal regulated pathway
GSK-3: Glycogen Synthase Kinase-3
HCY: homocysteine
HDAC: Histone Deacetylase
HDL: High-density Lipoprotein
MET: methionine
miRNA: MicroRNA
mRNA: Messenger RNA
NFT: Neurofibrillary Tangle
NMDA: N-Methyl-D-Aspartate
piwi: P-element–induced wimpy testis
piwi-RNAs: P-element–induced wimpy testis small RNAs
SAH: S-adensoyl homocysteine
SAM: S-adenosylmethionine
INTRODUCTION

Overview of Epigenetics

The recent expansion of epigenetics as a field of interest has provided researchers a novel avenue to approach regulation and manipulation of genetic material (Sweatt, 2013). Epigenetics is characterized by the manipulation of genes at both the transcriptional and post-translational level, meaning that this is active regulation of a gene occurring largely in response to external stimuli without altering the underlying DNA sequence (Xu et al., 2012). This sort of regulation allows for gene expression to be crafted to the specific needs of the body without manipulating the DNA transcript (Goldberg et. al 2007).

The most powerful epigenetic regulator is the methylation of the 5’ cytosine in a strand of DNA. Transcription is weakened if a gene contains a methylated cytosine in a characteristic location of the DNA (Sweatt, 2013). Another epigenetic regulator affects the chromatin structure by modifying aspects of the comprising histones (Fischer, 2014). Lastly, the most recently discovered method of epigenetic regulation is done by segments of non-coding RNA molecules, primarily microRNA (miRNA) (Sweatt, 2013). They are thought to have a gene silencing effect through disruption of the translational process at the level of the messenger RNA (mRNA) (Kannan and Ravi, 2013). The cumulative effect of these mechanisms provides for an extensive and responsive regulatory avenue for genes to be activated or silenced (Figure 1)(Wang et al., 2013).
In non-neuronal epigenetic regulation, there is no mechanism more powerful than methylation at cytosine-guanine rich sequences (CpG islands) (Sweatt, 2013). In neuronal epigenetic regulation, methylation can occur at any of the following dinucleotide variations: CG, CA, CC, or CT (Rudenko and Tsai, 2014). Normally, methylation is irreversible in the body as it is a covalent modification to the DNA sequence. Epigenetic regulation can be reversed in areas of the body that undergo adaptive changes through the life cycle, such as the aging nervous system and the maturation of pluripotent stem cells (Kannan and Ravi, 2013). Demethylation in the nervous system is extremely problematic because epigenetic regulation of neurons is critical to proper functioning of the cognitive processes (Rudenko and Tsai, 2014). In studies, methylation has been shown to occur at loci that are genetically and evolutionarily conserved (Xin et al., 2011). Therefore, changes in the longstanding sites of methylation by demethylation usually results in disease and dysfunction.
Figure 1. Mechanisms of Methylation. Figure shows that methylation occurs under the activity of DNA methyltransferase (A) and the effects of methylation versus unmethylated DNA (B). Ultimately, methylated DNA, in non-neuronal genes, will be silenced while transcription occurs uninterrupted in unmethylated DNA (Ho and Tang, 2007).

The second most prominent form of epigenetic regulation is through the modification of chromatin structure, specifically by chemically modifying histones (Wang et al., 2004). Various mechanisms of epigenetic regulation occur on the histone protein, particularly at the amino or carboxy termini of the protein (Maze et al., 2013). These modifications to the histone protein function to alter the charge associations between the protein and the surrounding coiled DNA (Goldberg et al., 2007). Through a number of distinct enzymes, histone tails can be modified by the addition or deletion of acetyl, methyl or phosphate groups (Borelli et al., 2008). These modifications regularly
occur in the maturation of the nervous system in order to accordingly develop long-term
memory and the ability to alter and amend behavioral mannerisms (Maze et al., 2013).

Proper maturation and development of the brain relies on regulation of pluripotent
stem cells by miRNAs (Barry, 2014). During the highly plastic years of neural
maturation, the role of miRNAs becomes reduced to accommodate abundant protein
synthesis necessary to facilitate the millions of synaptic connections that will be formed
(Krol et al., 2010). Although non-coding RNA activity is reduced, the activity present is
essential to enhancing memory development and ideal cognition. It has been shown that
miRNAs and piwi-RNAs (piwi: P-element–induced wimpy testis small RNAs) strengthen
the ability of the synapse to transfer neural messages by blocking protein translation that
would confer synaptic inhibition (Barry, 2014). However, when non-coding RNAs are
either over-expressed or hyperactive, neurological deficit and cognitive dysfunction could
result. Messenger RNA molecules carrying transcripts for essential proteins could be
targeted for degradation resulting in a deviation from normal neural activity (Abe and
Bonini, 2014).

Overview of Alzheimer’s Disease

Alzheimer’s disease (AD) is the leading cause of neurological deficit in people
aged 65 and older, while also affecting younger people (Thies et al., 2013). The
expenditures of treating this disease and caring for affected patients have skyrocketed in
the past 50 years (Figure 2)(Thies et al, 2013). The increased cost is due to the incidence
of this condition rising so dramatically, from just under 6 million diagnosed cases today
to a projected 12 million cases in the next 30 years (Defina et al., 2013). When AD affects people under the age of 65, it is referred to as early onset AD whereas age 65 and older is referenced as late onset AD (Panegyres and Chen, 2013). Aside from the age implications, the major difference between these two subsets of AD is that early onset AD occurs due to autosomal dominant inheritance, while late onset AD occur due to a plethora of genetic and environmental factors (Nygaard et al, 2014).

Figure 2. Expenditures attributed to AD in 2013. As displayed above, $203 billion dollars was spent in the year 2013 to treat and provide care for patients affected with AD. About 70% of the total cost was covered under Medicare and Medicaid, but that still leaves 30% of $203 billion dollars that was paid either out of pocket or by private insurance. This figure demonstrates the financial burden of AD (Thies et al, 2013).
Early onset AD accounts for a minute proportion of the total number of cases of AD, but has devastating effects due to the inheritable nature of this subset of AD (Roher et al., 2013). Early onset AD is characterized by an accelerated progression of AD starting in patients as early as in the early 40’s versus late onset AD starting after age 70 (Panegyres and Chen, 2013.) The early onset cases tend to show rapid rates of memory loss and also tend to show augmented deficit histopathologically (Panegyres and Chen, 2013). Mutations in presenilin 1, presenilin 2, and amyloid precursor protein (APP) are considered to be causative of early onset AD (Nygaard et al, 2014). Presenilin 1 gene mutations comprise a majority of the cases of early onset AD (Sproul et al., 2014). The presenilin 1 gene codes for the enzyme gamma secretase, preventing normal processing of APP (Chen et al., 2006). A mutation in the presenilin 1 gene results in defective APP processing leading to accumulation of beta amyloid (Aβ) (Sproul et al., 2014). Mutations in the presenilin 2 and APP genes are also thought to cause impairments in the processing of APP, but these mutations are less prevalent than the aforementioned presenilin 1 mutation (Armstrong, 2014). If these causative genes are altered, the subsequent amyloid deposition is enhanced and is thought to provide the basis for the progression of AD (Weggen and Beher, 2014).

The diagnoses of late onset AD comprise an overwhelming majority of cases of AD in the United States (Thies et al., 2013). The course of this AD subset is less clearly understood as compared to early onset AD (Panegyres and Chen, 2013). Early onset AD has three specific causative genes, while late onset AD is multi-factorial with both genetic and environmental factors being suggestive as causative factors (Balin and
Hudson, 2014). Although there has not been evidence of a causative gene in late onset AD, the apolipoprotein E (ApoE) gene has been shown to be associated with a considerable amount of late onset AD cases (Armstrong, 2013). ApoE encodes one of three significant versions of the ApoE protein (APOE): APOE2, APOE3, and APOE4 (Hudry et al., 2013). These proteins all function to modulate the activity of Aβ by processing it accordingly as Aβ is produced. However, APOE4 is far less efficient than the other two proteins in controlling the deposition of Aβ (Jiang et al., 2008). Therefore, APOE4 acts as a specific risk factor for late onset AD even though cases of AD can appear without the presence of APOE4 (Hudry et al., 2013). Since there is no explicit singular cause for late onset AD, a definitive diagnosis is difficult to ascertain prior to an autopsy (Balin and Hudson, 2014).

The only effective means of diagnosis that is available is an examination of the cerebrospinal fluid of a patient experiencing dementia-related symptoms; the fluid is examined for specific Aβ levels and elevated tau protein levels (both phosphorylated and unphosphorylated) (Figure 3)(Randall et al., 2013).
Figure 3. Biomarkers of AD in Cerebrospinal Fluid. The flow diagram above shows the extrapolation that can be made if a certain biomarker is found to be in an aberrant range in the cerebrospinal fluid. Specifically, if Aβ42 (the normally processed version of APP) is low or phosphorylated tau (in diseased state) is high, then it can be inferred that the physical and behavioral symptoms of AD should also be expected (Randall et al., 2013).

Pathogenesis of AD

AD is a hallmark neurodegenerative disease characterized by two major dysfunctions in normal neural metabolism: first, the inability to properly process the APP and second, the inappropriate phosphorylation of tau proteins (Xu et al., 2012). Dysfunctional APP processing results in the formation of plaque depositions composed of beta-amyloid (Aβ), while hyperphosphorylated tau proteins aggregate to form
neurofibrillary tangles (NFT) (Balin and Hudson, 2014). Although, the complete pathology of AD is still to be uncovered, it seems the failed synaptic transmission and neuronal loss can be attributed to the inappropriate aggregates (Wang et al, 2013).

Cleavage of APP normally occurs by alpha secretase followed by gamma secretase. This enzymatic succession however, can be altered by the substitution of beta secretase for alpha secretase (Chen et al., 2006). This replacement results in the formation of Aβ deposits, which can be metabolized, but if deposition is greater than clearance rate then plaque formation is initiated (Kim et al, 2009). NFTs form in the neuronal cytoplasm due to the hyperphosphorylation of the cytoskeletal protein, tau (Lee et al., 2005). The tau protein allows for axonal transport by providing microtubule stability, mainly through phosphorylation of the protein itself (Bloom, 2014). However, hyperphosphorylation of tau shifts it from its normal soluble state to an insoluble form, which favors aggregation (Jayapalan and Natarajan, 2013). The trigger for this aberrant phosphorylation seems to be the deposition of Aβ into extracellular plaques; the succeeding hyperphosphorylation provides positive feedback to Aβ causing the plaque formation to be exacerbated (Bloom, 2014). See Figure 4 below for a visual representation of the cumulative effects of ApoE and APP processing:
Figure 4. Potential causes of AD progression. The occurrence of AD is strongly linked to aberrations in the ApoE gene. If there is an alternative isoform of ApoE, the resulting dysfunction would result in aggregation of the Aβ. However, clearance of this protein is affected if ApoE is lipidated. The combined effects of the many defects result in the progression of AD (Kim et al., 2009).

AD and Co-Morbidities

The availability of glucose and adequate blood flow to the arterioles in the cortex of the brain are critical to proper neurological function (Popa-Wagner et al., 2013). Both of the requirements to maintain normal neural processes are not usually upheld in cases of AD (Popa-Wagner et al., 2013). Cerebrovascular disease and AD are generally co-morbidities, even though they are separate conditions (Lee et al., 2014). The reason for
this co-occurrence relies on the fact that deposition of the Aβ is not solely restricted to the extracellular plaques of AD or the media of the cerebral vessels as in cerebrovascular disease (Honjo et al., 2012). Deposition of Aβ in the walls of vessels generally results in the early apoptosis of endothelial cell, and therefore collapse of associated blood vessels (Armstrong, 2013). The impaired integrity of blood vessels causes a resultant disruption in the continuity and strength of the blood-brain barrier (Armstrong, 2013). Both of these conditions rely on the impairment on Aβ/APP processing, which is why they coincide in a majority of cases.

The oxidative stresses and vascular implications undergone by normal aging can become risk factors for the impending development of AD. One serum protein that has been shown to be a risk factor for AD is high-density lipoprotein (HDL); low levels of HDL have been correlated in the pathogenesis of AD (Stukas et al., 2014). The main function of HDL is to bind cholesterol and disallow it from being free in blood. If HDL levels in blood are inadequate, then serum cholesterol levels will rise (Reed et al., 2014). Excess cholesterol can be deposited into vessel walls, and over time the hardened arteries can chronically raise blood pressure, a condition known as hypertension (Grammas et al., 2014). The neural effects of hypertension result in reduced cerebral blood flow, and the aforementioned effects of hypo-perfusion to the brain (Skoog and Gustafson, 2006).

A recent study by Huang et al. was conducted to examine the relationship between AD and diabetes mellitus; this study used approximately 142,000 people from a random sample of one million Taiwanese people (Huang et al., 2014). Of these 71,000 were subjects that had diabetes mellitus while the other 71,000 were subjects that did not
have the condition. These two groups were tested against one another to measure the occurrence of AD. The results of the study displayed that the diabetic subjects had a higher occurrence of AD, and therefore a greater risk for developing AD (for those diabetic subjects that didn’t have it) (Huang et al., 2014). Diabetes mellitus increases the risk for AD in a similar manner as hypertensive conditions do (Moreira et al., 2013). Diabetes mellitus has been shown to present with increased levels of triglycerides, free fatty acids, visceral fat, insulin resistance, and oxidative stress (Hanyu, 2014). The increased levels of triglycerides and free fatty acids are a result of the low HDL levels. They eventually deposit into vessel walls and cause localized oxidative stress (Skoog and Gustafson, 2006). The cumulative effect of numerous depositions results in an overall excess of oxidative stress leading to impairment of cognitive function (Hanyu, 2014).

In addition, insulin and insulin resistance play major roles in increasing the risk of developing AD. The memory and learning centers of the brain are coated in insulin receptors to mediate the amount of glucose that neurons in that region of the brain uptake (Craft, 2009). In a diabetic patient, insulin receptors experience a degree of resistance that disallows insulin binding, and in effect the uptake of glucose into cells. If the neurons cannot get enough glucose, they cannot properly function or transmit information. Lastly, insulin has been linked to the increasing the rate of Aβ deposition, both directly through the processing of APP and through the increased phosphorylation of tau, which has a positive feedback effect on Aβ deposition (Moreira et al., 2013). Normally, the insulin receptor acts through the phosphoinositide 3-kinase pathway, which has a downstream effect of inactivating a major protein implicated in the deposition of Aβ and the
hyperphosphorylation of tau. However, in a patient with diabetes mellitus, the insulin resistance prevents insulin from occupying the insulin receptor. The insulin receptor can then bind Aβ, which prevents the progression of the phosphoinositide 3-kinase pathway resulting in overactive deposition of Aβ and phosphorylation of tau (Salcedo-Tello et al., 2011).

Current Therapeutic Avenues for AD

Cholinesterase Inhibitors

The overwhelming worldwide presence of AD has constituted a dire need for effective therapeutics. More than half a trillion dollars was spent in an effort to alleviate this condition by the year 2010 (Corbett et al., 2013). Even though a tremendous amount of money was allocated in efforts to decrease the prevalence of AD, the incidence of this disease keeps rising steadily (Thies et al., 2013). It can be assumed that if the costs keep rising with no decrease in prevalence, then the efficacies of the current treatments are insufficient. The lack of effective speaks volumes regarding the complexity of AD and its multiple mechanisms of pathology.

AD is a neurodegenerative disease; the brain will experience cortical atrophy, which induces the symptoms of this condition (Serrano-Pozo et al., 2011). The current medications in widespread use do not address the pathology of the disease; rather they act to alleviate symptoms (Corbett et al., 2013). The major category of medication that is used to treat the symptoms of AD is cholinesterase inhibitors (Birks, 2006). This style of
medication is used to treat the cholinergic impairment that is generally indicated in cases of AD (Birks, 2006). Acetylcholine (Ach) is the primary mode of signal transmission in the brain; over-active cholinesterase decreases the ability of Ach to transmit signals resulting in global neurological deficits (Anand and Singh, 2013). Reception of cholinergic signals is highly imperative in the process of learning and retention; the major pathway that the hippocampus and cortex receive signals is through cholinergic neurons (Hatayama et al., 2014). Therefore, inhibition of acetylcholinesterase is effective in allow for levels of Ach that are potent enough for the forebrain to transmit signals to the learning and memory centers. Additionally, cholinesterases have been indicated in the progression of AD pathogenesis, such that stunted levels of Ach results in increased Aβ plaque formation (Anand and Singh, 2013).

Tacrine was the initial attempt at bringing a cholinesterase into the market to actualize the effects of increasing the availability of Ach for patients suffering from mild to moderate AD. However, it was deemed ineffective and removed from the market due toxic side effects to the liver (Birks et al., 2009). To improve on the drug Tacrine, researchers produced several highly specific cholinesterase inhibitors to decrease the chances of adverse toxicity. These drugs are currently used in the treatment of AD and include Donepezil (Aricept®, Pfizer), Rivastigmine (Exelon® Novartis), and Galantamine (Razadyne®) (Anand and Singh, 2013). These cholinesterase inhibitors have been proven in clinical studies to alleviate some of the behavior symptoms, memory problems, and general awareness of AD patients (Birks, 2006).
Memantine (Namenda® Forest Laboratories) is a drug used either as a standalone or coupled with the administration of a cholinesterase inhibitor (Molino et al., 2013). N-Methyl-D-Aspartate (NMDA) is a prominent type of receptor in the brain involving the memory and learning centers (Molino et al., 2013). The NMDA receptor is activated by increased levels of glutamic acid, which is overproduced in cases of neurotoxicity, causing an unprecedented amount of calcium to influx into the cells resulting in cell lysis (Rosini et al., 2014). In a patient with AD, glutamic acid levels are higher and cause over-activation of NMDA receptors. The function of Mementaine is to bind to the NMDA receptors so that they cannot recognize the elevated glutamine acid levels, thereby preventing unnecessary cell death (Lipton, 2005). Although the efficacy of Memantine has been verified, the cocktail of Memantine and Donepezil (or any other cholinesterase inhibitor) has not been shown to provide marginally better results than either of the drugs acting alone (Molino et al, 2013).

**The Tau Model of Treatment and Kinase Inhibitors**

The hypothesis of Aβ deposition leading to the full-fledged pathogenesis of AD is referred to as the “amyloid cascade hypothesis” (Hong et al., 2014). The fact that AD pathogenesis has not yet been definitively understood has led researchers to explore other avenues of pathology and treatment. Largely, the treatments derived from the pathogenesis presented from the amyloid cascade hypothesis have been only modestly effective, in that they only treat superficial symptoms (Takata, 2013). Some researchers
have been making the effort to explore the role of the tau gene and how its pathogenesis can be evaluated and treated.

The tau protein is of paramount importance in the pathological mechanisms leading to the neurotoxicity present in AD. It has been demonstrated that a simple mutation in the tau gene, without any Aβ deposition, can provide levels of toxicity that would result in dementia (Medina and Avila, 2014). Therefore, it is evident that mutations in the tau gene, or dysfunctions in the tau protein, can result in significant overall neurological impairment. These mutations usually result in the formation of a tau isoform that favors fibril formation over the normal function of stabilization of microtubules (Brunden et al., 2010). Similarly, hyperphosphorylation of the tau protein causes the tau protein to aggregate in the same fashion even though gene mutations regarding the tau isoforms have not be identified in the pathogenesis of AD (Brunden et al., 2010). Hyperphosphorylation of the tau protein promotes the fibril aggregation due to the phosphates on the tau protein physically preventing the binding domains of the tau protein from interacting with the microtubules (Figure 5)(Pevalova et al., 2006).
Figure 5. Effects of the phosphorylated Tau protein on normal interactions of Tau. Normal unphosphorylated tau protein interacts with microtubules to stabilize. However, when phosphate groups are attached to the tau protein, there is steric hindrance, which disallows binding to microtubules causing two effects: the phosphorylated tau proteins will tend to aggregate and unstable microtubules will cause disrupted axonal transport (Kolarova et al., 2012).

Thus, to attack the issue presented in AD, kinase inhibitors are thought to be an effective therapeutic intervention because tau phosphorylation can potentially be controlled (Medina and Avila, 2014). Since the tau protein is hyperphosphorylated in both the early and late onset subsets of AD, inhibiting kinase activity preventing phosphorylation is thought to provide for a productive avenue of treatment.

A prominent kinase that is abundantly present and overactive in the pathology of AD is the glycogen synthase kinase-3 (GSK-3) (Ma, 2013). GSK-3 is suggested to both partially participate in the phosphorylation of tau itself and is indicated in the impaired
processing of APP (Brunden et al., 2010). In a mouse model, the inhibition of an isoform of GSK-3 (GSK-3b) produced a reduction in Aβ deposition (Ly et al., 2013). Over-activation of GSK-3 results in the downstream signaling that increases expression of the gene (BACE1 or beta-site amyloid precursor protein cleaving enzyme 1) that transcribes the beta secretase enzyme (Figure 6)(McConlogue et al, 2007). It has been observed in transgenic mice models that reduced BACE1 expression has led to a decrease in the rate of Aβ deposited due to less beta secretase present (Vassar, 2001). It is important to mention that the BACE1 expression is affected by GSK-3 activity through the NFκB signaling pathway (Ly et al., 2013). Inhibition of the upstream kinase activity at the GSK-3 is promising as an avenue for targeted therapy to shut down the NFκB signaling pathway. GSK-3 activity can also be inhibited through the addition of phosphates at particular locations on the GSK-3 protein (Ly et al., 2013). The serine residues on GSK-3 (both the GSK-3a and GSK-3b isoforms) are the susceptible locations for GSK inactivation; serine residue phosphorylation results in the reduction of GSK activity (Figure 6)(Hooper et al., 2008).
Figure 6. Effects of GSK on Aβ deposition and the hyperphosphorylation of tau proteins. This diagram shows how the initial Aβ compounds can both inhibit the inactivation of GSK-3 protein and potentiate GSK-3 activity, concurrently. Aβ prevents inactivation of GSK-3 by disallowing the normal progression of the phosphoinositide 3-kinase pathway (which normally results in the phosphorylation of the serine 9 residue.) Aβ amplifies GSK-3 activity by providing more tyrosine activation through a signaling pathway initiated by the Aβ oligomer (Salcedo-Tello et al., 2011).
Centrally-acting Angiotensin-I Converting Enzyme Inhibitors

This class of inhibitors target the Angiotensin-I Converting Enzyme (ACE), which converts angiotensin I to angiotensin II in an effort to engage the renin-angiotensin-aldosterone system to regulate blood pressure. Some studies have shown, although not definitively, that over-activity of centrally-acting ACE (and therefore the renin-angiotensin-aldosterone system) leads to impairment of neural processing (Sink et al., 2009). Therefore, the proposition of inhibition of centrally-acting ACE inhibitors came about. In a study by O’Caoimh and colleagues, they showed that centrally-acting ACE inhibitors, along with their expected anti-hypertensive effects, reduced the rate of cognitive decline by a considerable amount (O’Caoimh et al., 2014).

Cardiovascular Exercise

There is an existing longstanding correlation between regular cardiovascular exercise and salubrious neurological function. The mechanisms underlying this relationship remain to be elucidated, but it is currently perceived to be as a result of the beneficial effects exercise exerts on vascular health (Tarumi and Zhangs, 2014). Aerobic activities can be used as preventative measures against AD because they can help improve the many implications that may result in the progression of AD, such as hypertension, hypoperfusion of the brain, visceral fat, and high free fatty acid levels that may lead to atherosclerosis (Jedrziewski et al., 2014).
SPECIFIC AIMS/OBJECTIVES

Epigenetics presents a promising method of targeting and treating the neurological deficits of Alzheimer’s disease. It allows for the risk genes and factors to be assessed and possibly manipulated for prevention of symptoms and pathogenesis of the disease. However, there are only a few concrete risk genes that can be targeted and these genes may not be the same in every case. What forms of therapeutic intervention can we provide to address and manipulate risk factor genes, and how can we ensure that patient’s receive a targeted treatment rather than standardized medicine?

Through the proper assessment and analysis of epigenetic mechanisms and the current dysfunction in the pathogenesis of Alzheimer’s disease, we hope to demonstrate the potential value of epigenetic therapeutics for Alzheimer’s disease treatment and prevention. The main goals of this study are to:

1. Understand the role of epigenetic regulation in neural processing and how it affects the maturation of the nervous system.

2. Learn the current and potential uses of epigenetic therapeutics through analysis of recent trials and determine its impact on personalized medicine.

3. Apply knowledge of epigenetic regulation to propose new avenues of treatment of patients suffering from Alzheimer’s disease.

Accomplishing these goals could lead to vast advancements in treating AD because currently the treatments are largely ineffective, and these goals provide a way for patients dealing with AD to have a long-term better quality of life.
Epigenetic Regulation in Neurogenesis and Neural Processing

During the process of neurogenesis, the stem cells allotted to the nervous system are differentiated into their mature forms. The process of maturation, from undifferentiated to differentiated, in a majority of cases relies on if a precursor cell is methylated or not (Kannan and Ravi, 2013.) A specific example of this in the developing nervous system is the differentiation of neural stem cells to astrocytes: if the precursor is not methylated in a certain domain, then the differentiation proceeds as an astrocyte whereas if the domain is methylated, the precursor becomes a neuron instead (Steoguchi et al., 2006). It has been shown that mature neurons express many methyl-binding domains, which rectifies this suggested process of methyl dependent maturation (Steoguchi et al., 2006).

According to Day et al., the methylation of DNA, excluding the promoter region, results in enhanced long term potentiation and memory formation (Day et al., 2013). A 2006 study by Levenson et al. showed that low levels of methylation in the hippocampus impaired recall of learned information and synaptic transmission, but the ability to process new information was retained (Levenson et al., 2006). DNA demethylation usually occurs when neurons are undergoing mitosis, but should maintain a methylated status post-division (Feng and Fan, 2009). Therefore, it can be assumed that a decreased level of methylation in a post-mitotic state is indicative of some degree of neurological
deficit (Guzman-Karlsson et al., 2014). Methylation occurs due to the activity of DNA methyl transferases (DNMT), and it has been shown that the levels of methylation (as a direct result of inactive DNMT enzymes) decrease as an individual ages (Wang et al., 2013).

Modifications of histone side groups are of significant import in the functions of normal cognition and retention of information. The N-terminal lysine residue is the target of various histone modifications, but the most important to regulation of the neural processes is acetylation (Sweatt, 2009). The lysine residue is the optimal target because it provides a favorable charge association between the positively charged lysine and the negatively charged acetyl group (Tanner et al., 1999). Just as methylation requires a specific donor group to donate the methyl, histone acetylation requires the compound acetyl coenzyme A to donate an acetyl group to allow the enzyme, histone acetyltransferase, to properly function (Tanner et al., 2000). Additionally, histone acetylation is a requisite process for DNA transcription to occur because the DNA needs to be uncoiled from the histone (the charge associations between the histone and DNA need to be weakened) (Figure (Wang et al., 2013). This process of acetylation is highly implicated the transcription of DNA and in the hippocampus in the process of memory retention, as a result of conditioning by fear (Rudenko and Tsai, 2014).

It has been shown in several studies that behavioral conditioning on the basis of aversion occurs when levels of acetylation and phosphorylation are increased on a specific histone, H3 (Rudenko and Tsai, 2014, Chwang et al., 2006, Lubin and Sweatt, 2007). This process of acetylation can be reversed in the central nervous system by a
class of enzymes known as histone deacetylases (HDAC) (Sweatt, 2009). Deacetylation is a normal process, but can be deregulated to the point where the acetylated H3 levels are too low to provide the optimal conditions for forming contextual fear memory retention. It has been postulated that the ERK/MAPK signal transduction pathway is implicated in regulating the levels of acetylation, where activation of extracellular signal regulated pathway (ERK) results in the acetylation of H3 (Chwang et. al 2006). On the H3 histone, it has been demonstrated that phosphorylation of the serine residue prior to the activation residue (lysine) can lead to an activation of transcription (Figure 7)(Day and Sweatt, 2011). However, a recent study on the role of this pathway in the modification of histones has brought this connection into question through elucidating several cases where ERK activation and acetylation/phosphorylation were found to be independent of one another (Ciccareli and Giustetto, 2014). Despite the controversy over the regulation, the fact remains that high acetylation levels and phosphorylation levels coincide with the formation of contextual fear memories.
Figure 7. Effects of Histone Modification on Transcription. Methylation of the histone tails leads to suppression of transcription because the methyl groups sterically hinder the acetyl groups from activating transcription (a). The effects of modifications on different residues of the H3 histone are displayed (b). Methylation of the H3 Lysine 9 residue may be the cause of transcriptional repression whereas activation can occur due
to acetylation of the H3 Lysine 14 residue or phosphorylation of the H3 Serine 10 residue (Day and Sweatt, 2011).

Non-coding RNA molecules include various types of RNA, but the main non-coding RNA of focus in neuroepigenetics is the miRNA (Barca-Mayo and De Pietri Tonelli, 2014). miRNAs are extremely small sequences of nucleotides that function to selectively express some genes while inhibiting others through blocking mRNA translation (Tan et al., 2013). miRNAs are thought to have an extensive role in the development of the nervous system (Mehler and Mattick, 2007). An experiment conducted by Giraldez et al. using a zebrafish model showed that zebrafish embryos that were devoid of miRNAs could not develop the fundamental aspects of even an embryonic nervous system (Giraldez et al., 2005). miRNAs are also implicated in the differentiation of neural stem cells into either neurons through neurogenesis or glial cells through gliogenesis (Mehler and Mattick, 2007).

miRNAs are single-stranded RNA molecules that function as part of the RNA-induced silencing complex, which allows for selective repression of translation through targeting specific mRNA (Hebert et al., 2012). Mature miRNA molecules are processed versions of precursor RNA molecules, which have been cleaved by the enzymes dicer and Drosha (Hugon and Paquet, 2008). A 2008 study conducted by Baek et al. was able to further elucidate the nature of miRNA by distinguishing that one miRNA strand (associated with its RNA-induced silencing complex) could control the expressions of hundreds of proteins through mRNA translation inhibition (Baek et al., 2008). Several studies have already established that miRNA deregulation is a component of dysfunction
in various neurological and cancerous conditions (Mehler and Mattick, 2007). Due to this fact, Cogswell et al. have proposed a method of identifying neurological diseases, AD specifically, through analysis of cerebrospinal fluid for abnormal levels of miRNA expression/regulation (Cogswell et al., 2008). miRNA targeted therapeutics are currently undergoing extensive research in various cancer conditions, but the expansion of this avenue in neurological disorders will provide for a method of attack the disease before the onset of progression through the use of biomarkers and correction of miRNA deregulation.

**Epigenetic Therapeutics and Personalized Medicine**

The most renowned cases using epigenetic mechanisms as therapeutics are in the treatment of various types of cancers. It has recently been identified that various epigenetic mechanisms are at the root of the tumorigenesis (Timp and Feinberg, 2013). A particular modification that has been observed is that the promoter regions of tumor suppressor genes (the areas of the genome that will enable transcription to occur) become silenced through methylation; silenced tumor suppressor genes lead to dysregulation of growth and proliferation (Timp and Feinberg, 2013). The cancer-infected gene’s chromatin is also compromised due to the dysregulation of the enzymes that regulate chromatin (Mair et al., 2014). The combined impairment of these two mechanisms leads to the full-blown instability and exponential proliferation of tumors characteristics. With the pathogenesis of cancer being exposed as having a strong epigenetic component, the interest in epigenetic therapeutics became discussed with more vigor.
As previously discussed, DNA methylation is the foremost method of epigenetic regulation in the human body. Methylation of a DNA sequence prior to transcription occurs due to a family of DNA methyl transferases (Adwan and Zawai, 2013). For the DNMT to successfully transfer a methyl group to a CG-rich region of DNA there needs to be a methyl donor group. The most common methyl donor group is the S-adenosyl methionine (Liu et al., 2009). Once the S-adenosyl methionine group donates its methyl group, the resultant products are homocysteine and adenosine. It has been observed that the amount of homocysteine in the blood of a patient affected with AD is abnormally high. This observation seems to indicate that some aspect of the process of methylation is aberrant in AD patients because homocysteine and adenosine would normally coalesce again in the presence of methyl (Zawia et al., 2009).

Currently, the approved and actively used forms of treatment regarding DNA methylation are in cancer-patients. In certain cases of cancer, the use of a DNMT inhibitor is extremely beneficial because it can halt the rampant over-expression characteristics of dysplastic disorders by inhibiting transcription (Xu et al., 2012). Specifically, DNA needs to be methylated on the daughter strand to identify which strand needs to be copied, and if that methylation is not present, transcription halts. By disallowing transcription to occur so rapidly, the exponential cell growth that is present in all forms of cancer can be controlled to a degree. There are some DNMT inhibitors undergoing clinical trials for approval, but most of these drugs are still being investigated on animal models (Xu et al., 2011).
The maintenance of acetylation levels in the hippocampus is essential to influencing behavior and learning processes (Rudenko and Tsai, 2014). One of the major problems with maintaining acetylation at an optimal level is the aberrant action of HDACs in neurodegenerative disorders (Sweatt, 2009). There are several classes of HDACs that function both in normal memory formation and in the prevention of neural processing by reducing long-term potentiation and memory formation (Fischer et al., 2010). A specific HDAC that seems to be implicated in AD is HDAC2; in an autopsy of an AD patient, the levels of this enzyme were found to be higher than the other HDACs (Graff et al., 2012). To target the HDACs, research into the inhibition of HDAC was conducted. HDACs are drawn to the N-terminal histones through various mechanisms of epigenetic origin (methylation more-so than others) leading to the chemotactic attraction between the HDAC and target site (Martinowich et al., 2003).

One of the most common epigenetic treatments in practice is the use of HDAC inhibitors to potentiate the cognitive processing involved in the formation of memories (Guan et al., 2009). An experiment was conducted in a mouse model and found that a HDAC2 inhibitor resulted in increased ability to learn and recall (Guan et al., 2009). This finding implies that the overexpression of HDAC2 (or the specific reduced acetylation at the target site) results in impairment of synaptic transmission (and thereby recall of information.) This study also displays the fact that there is a certain degree of selectivity in deacetylation and its effect on memory; HDAC2 appears to have significantly higher impact on impairment of memory formation than HDAC1 does in animal models (Guan et al., 2009.)
A recent study has further demonstrated the importance of HDAC inhibition using another mouse model also. Selenica et al. explored the effect of HDAC6 inhibition on tau pathology. The findings of this experiment demonstrated that not only was the inhibition of HDAC6 important in restoring learning process, but that the inhibition also showed positive effects in decreasing the amount of tau deposition (Selenica et al., 2014). As stated in prior sections, the tau protein is vital in axonal transmission by allowing for stabilization of microtubules. Therefore, the essential reduction of defective tau may yield to a very promising effect on learning and memory.

With the possibility of epigenetic mechanisms being implicated in treatment, the subject of using epigenetic markers in the identification of disease has become more prevalent. In a study conducted by Cheng and Chiang, the use of an epigenetic predictor was tested to ascertain whether assessing sites of aberrant methylation was a viable approach to determine if a specific cancer was present (Cheng and Chiang, 2013). Although the study concluded that use of predictors to identify a specific tumor or disease was impractical, the results did show that the findings from a predictor could be useful in ascertaining which follow-up tests to proceed with in order to accurately and promptly diagnose patients (Chang and Chiang, 2013). The availability of such mechanisms opens up a world of possibilities for the accurate diagnosis of neurodegenerative disorders, specifically AD.

Inter-individuality between the epigenetic mechanisms in one person versus another is of tremendous variability (Goldberg et al., 2007). To elaborate, epigenetic mechanisms rely on environmental and internal stimuli to express a response. In a normal
subject, with no underlying aberrant activity, epigenetic mechanisms allow for the response that is most beneficial for the individual with respect to internal and environmental conditions. This active approach to the progression or repression of gene transcription allows for individual adaptability. When the mechanisms of epigenetic regulation deviate from the norm to a degree that it leads to the progression of a disease, the diagnosis and treatment of the source of the disease become much more intricate.

With further elaboration and experimentation of predictor models, such as the one used in Cheng and Chiang’s study, accuracy in diagnosis based on an individual’s risk markers can be obtained (Cheng and Chiang, 2013).

**Epigenetics and Alzheimer’s Disease**

Early onset AD has been attributed to mutations in the APP-processing genes: presenilin 1, presenilin 2, and the APP (Nygaard et al., 2014). The only strong established genetic link for the late onset subset of AD is ApoE (Jiang et al., 2008). It should be noted that the ApoE gene displays the widest degree of aberrance in methylation patterns amongst AD patients (Zawia et al., 2009). A recent mass spectrometry study has shed more light on the possibility of a greater genetic component in the pathogenesis of sporadic AD. Through autopsy, the group of researchers identified altered methylation patterns in patients with AD based on age. It was concluded that as the age of the patient increases, the post-mortem examination showed further aberrance in methylation (Wang et al., 2008). The results of this study are compelling because the evidence suggests that...
genetics, specifically epigenetics, may play a much larger role in the progression of AD than previously expected.

The patterns of normal DNA methylation do not seem to be upheld during the pathogenesis of AD. Toghi et al. elucidated that aging is a factor in decreased levels of methylation (Toghi et al., 1999). However, the specific demethylation of APP promoter gene occurs to a great degree in the instance of AD rather than the lesser amount of demethylation due to normal aging (Toghi et al., 1999). Demethylation of the promoter allows for more APP to be transcribed and translated, leading to more processing (thereby a higher probability of dysfunctional processing in predisposed patients.)

Demethylation of APP is not the only factor in the epigenetic dysregulation that occurs during the onset of AD; deregulation of phosphorylation has also been indicated to play a role in tau pathogenesis. Tau pathology has been shown to begin with the phosphorylation of a threonine-231 residue (Luna-Munoz et al., 2007). Multiple pathways mediate tau phosphorylation, but protein kinase A pathway activation seems to play a larger role in threonine-231 specific phosphorylation (Sanchez-Mut. et al., 2014). A particular dual-specific phosphatase (DUSP22) has been implicated in the pathogenesis of AD; dysregulation of its normal activity allows for the potentiation of threonine phosphorylation. It has been further suggested by Sanchez-Mut et al. that methylation of the promoter region of DUSP22 could provide beneficial results in terms of decreasing tau phosphorylation (Sanchez-Mut. et al., 2014).

Histone acetylation, as discussed before, is a crucial part of maintaining the ability to actively open coiled DNA to allow for transcription to occur (Wang et al., 2013).
Although previous studies have displayed that aged animal models have decreased levels of histone acetylation, Zhang et al. conducted and confirmed that not only do humans experience the same decrease in histone acetylation, but the reduction is greatly accentuated by the pathogenesis of AD (Zhang et al., 2012). Over-activity of HDAC family of enzymes is generally the culprit in cases of vastly reduced acetylation. Additionally, it has been demonstrated that the levels of the brain-derived neurotrophic factor gene (a gene of vast import to memory and maturation of the brain) are decreased in AD due to the implications regarding histone modification (Walker et al., 2012). The underlying mechanism of this regulation is dependent on high levels of acetylation to activate the transcription of the brain-derived neurotrophic gene (Maze et al., 2013). The current method of attacking this problematic issue is by addressing the root of the decreased levels of acetylation. Therefore, the standard of treatment is to inhibit the HDACs to allow for levels of acetylation to remain at levels necessary for cognition, learning, and memory (Maze et al., 2013).

Recent years have shown an amassing amount of evidence linking alterations in miRNA action to the progression of AD (Tan et al., 2013). Studies have shown that various miRNAs have been observed to be at aberrant levels in patients with AD (Hugon and Paquet, 2008). In a study conducted by Lukiw, it was demonstrated that, in comparison to a fetal unaffected hippocampus, miRNA expression was altered in the AD hippocampus (Lukiw, 2007). Specifically, the fetal hippocampus levels of a multitude of miRNAs were measured. When these levels were compared to AD-affected hippocampus, the results showed that while the levels of the miRNAs were high in the
fetal hippocampus, the AD hippocampus showed augmented expressions of miRNA-125b, miRNA-128, and miRNA-9 (Lukiw, 2007). However, miRNA levels do not always increase in the pathogenesis of AD, miRNA-107 has been extensively studied and shown to actually decrease in AD (Wang et al., 2008b). Figure 8 further shows the implications of miRNA in the progression of AD (Tan et al., 2013).
Figure 8. The implication of aberrant miRNA regulation in AD. The process of miRNA production (left panel) is shown with the mature miRNA originating from a primary precursor, which is cleaved twice; once by Drosha within the cytoplasm and next by dicer outside of the cell. Dicer establishes the length of the miRNA (at around 20 NTs), but the double stranded nature of it disassociates and degrades naturally. Shown on the right are the various contributions by deregulated miRNA to the pathogenesis of AD. miRNAs can be implicated in tau toxicity, plaque deposition and accumulation, and vascular problems. They have also been indicated to cause early cell death, in few cases (Tan et al., 2013).
**Environmentally-influenced Epigenetic Changes in AD**

One of the most important characteristics of epigenetic regulation is that it comprises a category of mechanisms that are used in response to cellular, physical, and chemical stimuli; it is also a characteristic that leaves the host vulnerable to the environment. A heavily studied environmental component in the progression of AD is lead exposure (Zawia et al., 2009). It has been demonstrated that lead exposure in pre-disposed individuals carrying ApoE4 (the isoform of the ApoE gene that is implicated in late onset AD) induces a delayed cognitive decline (Stewart et al., 2002). The main proposed mechanism of lead’s influence on AD is that through an unknown process, lead leads to the upregulation of the mRNA that transcribes APP. Zawia et al. proposed that this upregulation could occur through the over-activation of a transcriptional factor (Zawia et al., 2009). This over-activation is related to the fact that the APP gene is largely methylated, and as a result lead is thought to demethylate the transcription factor of the APP gene. It has been observed that long-term exposure to the pesticides, such as dichlorodiphenyldichloroethylene and dichlorodiphenyltrichloroethane, produced the same effects of increased APP expression in pre-disposed individuals relative to those not exposed (Richardson et al., 2014).

For many years, oxidative stress was thought to be a product of the pathogenesis of AD or simply as a result of the co-morbidities that generally present with AD, such as hypertension, diabetes, and atherosclerosis (Moreira et al., 2013). Recent evidence suggests that oxidative damage is implicit in the early progression of the disease. Oxidative damage is usually seen in the same areas of a DNA sequence that methylation
generally occurs, the CpG island (Zawia et al., 2009). When a reactive oxygen molecule interacts with the CpG island, it preferentially binds to the guanine of the CG sequence to create an 8-hydroxyl-2’-deoxyguanosine (8-OHG). It has long ago been elucidated by Turk et al. that 8-OHG indirectly inhibits adjacent methylation of the cytosine in the CpG island by impairing the function of DNMT (Turk et al., 1995). More recently, the actions of 8-OHG have been implicated in AD. A 2007 study demonstrated that AD-affected brains displayed higher levels of 8-OHG, along with various other oxidized products, than aged-matched controls (Lovell and Markesbery, 2007.) This data is troubling because it shows a duality in the nature of oxidative stress and its impact on the underlying epigenetic mechanisms in the brain.

As mentioned above, oxidized products can inhibit the activity of methylation by reducing the functionality of DNMTs. However, reactive oxygen species can also oxidize the methylation target, cytosine in the CpG island, or even the methylated cytosine (Zawia et al., 2009). The grave quandary behind this oxidation is the deregulation of methylation. By essentially negating the methyl effect on the DNA, the transcription machinery will not receive signals to block the transcription of certain genes and allow for its full expression. In the case of AD, when the silencing effect of methylation on the APP gene is reduced, this leads to an over-expression of APP. Abundant APP leads to abundant processing of the protein, therefore the BACE1 gene will be upregulated as it one of the genes linked to APP processing (Vassar, 2001.) With increased BACE1 expression increased beta secretase activity results leading to amyloid deposition.
Furthermore, reactive oxygen species do not have to be produced or introduced in an excess amount for progression of AD to proceed. Evidence shows that regulatory functions limiting the amount of oxidative stress seem to be awry in cases of neurological deficit (Bolognin et al., 2014). There is a class of molecules in the body known as metallothioneins that function to provide protection against oxidation and metal damage. These proteins allow for binding of reactive oxygen species to preemptively disallow any oxidation damage that could have otherwise occurred. However, it has been discovered that metallothionein levels deviate from the norm during cases of neurological dysfunction, such as AD (Bolognin et al., 2014).

Nutrition also plays a significant effect in modulating the epigenetic changes associated with AD. The process of methylation and demethylation are inherently reliant on two major nutritional components: folate and choline (Liu et al., 2009). As described earlier, methyl is transferred from S-adenosyl methionine to a target, and the resulting homocysteine needs to be re-methylated for the methylation process to occur again. The process of remethylation cannot occur without adequate levels of folate and choline because these nutritional compounds provide additional methyl groups for the body to use (Figure 9)(Liu et al., 2009).
Figure 9. Methylation and Re-methylation Cycle. The relationship between folate/choline and the methylation cycle. SAM (S-adenosylmethionine) transfers a methyl group via the methyl transferase to a specific target. SAM will be converted homocysteine (HCY), which can revert to SAH (S-adenosyl homocysteine.) SAH will only remain in high levels if there is inadequate levels of methyl donors (folate and choline.) Inadequate amounts of folate and choline will prevent the conversion of HCY to methionine (MET), which will not undergo adenosylation to eventually reproduce S-adenosyl methionine (Liu et al., 2009).
A study conducted in 2013 further substantiated the effects of low level folate and methylation. Marzena and Jerzy showed that folate deficiency was highly evident in patients with AD whereas levels of folate were relatively normal or high in aged-matched control groups (Marzena and Jerzy, 2013). This correlation can be explained by the fact that deficiency of folate is sufficient to put the body in a globally hypomethylated state. Thereby, the normal silencing of the APP gene that occurs through the process of methylation is removed, and active transcription of this gene follows. The cascade of amyloid deposition and tau hyperphosphorylation may follow if enough time (even if it is many years) in this hypomethylated state is accrued.
DISCUSSION: PROPOSED THERAPEUTICS AGAINST EPIGENETIC Deregulation

AD is an irreversible neurological disease, which currently has no available therapeutic approaches that target the pathologic source of the condition. The therapeutics that are in mass production for this disease were contrived to provide relief from the symptoms. These treatments include, but are not limited to, various cholinesterase inhibitors and GSK-3 inhibitors (Birks et al., 2006 and Ma et al., 2013). Although these drugs are effective in alleviating some of the major symptoms of AD (such as long term potentiation, synaptic transmission, and even learning and memory formation), they do not treat the root of the symptoms (the underlying amyloid and tau pathology). One class of drugs that is currently undergoing trials on animal models is the HDAC inhibitor (Wang et al., 2013).

HDAC inhibitors have displayed tremendous potential as an epigenetic therapeutic for AD because they decrease both the deposition of Aβ and the hyperphosphorylation of tau (Wang et al., 2013). However, the current use of HDAC inhibitors is inefficient and of concern to the patient. These inhibitors are used as a general class of inhibitors that can target all HDACs rather than providing the specificity that is necessary when dealing with such an intricate condition as AD. There have been many reports of unintentional premature cell death regarding the use of non-specific HDAC inhibitors in cancer patients (Wang et al., 2013). Although there are some HDAC inhibitors, such as Tubacin, that target specific HDACs (Tubacin targets HDAC6), these
drugs need to be further investigated, as they are not approved for widespread use in humans yet (Ding et al., 2008). With further research into fine-tuning the specificity of these inhibitors, a powerful treatment for AD can be produced due to HDACs being able to provide an avenue to treat both aspects of the AD pathology.

DNA methylation is a powerful regulator of gene expression, but it is much more difficult to employ a method of treatment using this aspect of epigenetic regulation. Since the major deficit in AD is active methylation to keep AD-associated genes (such as APP, BACE1, presenilin 1 and 2) from being over-expressed, the use of DNMT inhibitors as a specific mechanistic treatment is not indicated. There are current investigations regarding hypermethylation processes in the progression of AD, but until there is solidified evidence of these processes, DNMT inhibitors do not pose enough benefit to warrant trials in AD patients (Wang et al., 2013). However, this does not preclude the use of methyl donors as a treatment option.

Methyl donors can help provide enough substrate for the methyl/re-methylation cycle to proceed undisrupted. These donors could be given to patient as part of a supplementation regimen or even in dietary changes, so long as the changes made in either supplementation or diet are long-term. It has been shown that in a double-blind, controlled study that elderly patients experienced an increase in cognitive function through a regimen of folate over the course of three years (Durga et al., 2007). While this method of treatment may not address the damage that has been done to the brain during the onset of AD, it can provide a method of halting or slowing the progression of AD by reducing one of the key pathologies, the hypomethylation. The additional methylation
could come from, but not limited to, the following sources: supplementation of S-adenosyl methionine, folate, choline, or even a potential DNMT analogue if the problem of hypomethylation has an enzymatic source.

As iterated before by many studies, miRNA deregulation has provided the avenue for progression of neurological disease (Abe and Bonini, 2013). Since miRNA molecules play a major role in neural processing by either allowing or disallowing the translation of various mRNA transcripts, these molecules make prime targets to enable the researcher to control expression of genes in a patient. The biggest challenge hindering the pathway of using miRNA as “switches” in gene expression is the actual delivery of miRNA analogues to target zones (Junn and Mouradian, 2012). Further research needs to be conducted on the use of miRNA as biomarkers to allow for genomic sequencing of an individual to provide a map, as to where regulation needs to occur. This will allow for a far more specific and individualized treatment plan for patients, catering to their particular genetic implication, rather than providing a standardized treatment that may or may not benefit them.

Another aspect of treatment that can be implemented is the down-regulation of over-expressed miRNA because some miRNA show aberrant action by providing over-activation of genes. For example, in a study conducted by Long and Lahiri, they found that reducing the expression of miRNA-101 actually produced a reduction in the amount of APP processing that was occurring in an AD animal model (Long and Lahiri, 2011). The problem with instituting this form of treatment in human patients is, again, the method of delivery. Kuhn et al. showed that anti-miRNA molecules are not able to cross
the blood-brain barrier on their own, leaving the only methods of transporting the anti-miRNA molecules to their targets are to either produce an apparatus to assist crossing the blood-brain barrier or direct injection into the brain (Kuhn et al., 2010). The latter poses dangerous consequences, so further research ought to be conducted exploring novel delivery mechanisms.

Lastly, an extremely effective and simple method of altering the epigenetic deregulation in the brain is for patients to institute a proactive and healthy lifestyle. The co-morbidities of AD not only lead to problems systemically, but also facilitate the progression of AD. These conditions affect the progression of AD by allowing the body to be in a state of high oxidative stress (Zawia et al., 2009). As previously discussed, oxidative stress impacts epigenetic regulation by preventing methylation at key sequences and allowing over-expression of genes that might otherwise be down-regulated or silenced (Vassar, 2001). A study conducted by Sardar et al. showed that aerobic exercise has provided great improvements in the treatment of diabetes mellitus type 2 by improving cardiac health and allowing patients to experience less symptoms of the disease (Sardar et al., 2014). The improved cardiac health in this condition could have tremendous benefits regarding cognitive function and AD. As noted earlier, there is a high incidence of cerebrovascular disease concurrently with AD (Popa-Wagner et al., 2013). One of the major implications of cerebrovascular disease is that the cerebral vessels get occluded and the brain subsequently becomes underperfused. Regular exercise, as it has been proved to improve cardiac health significantly by Sardar et al., would allow for higher perfusion pressures and potential increases in blood flow to the
brain. Increased flow to the brain can decrease the complications of cerebrovascular disease and provide neurons with the enriching nutrients that they need to function at a high level.
CONCLUDING REMARKS

Alzheimer’s disease is a devastating disease that needs to continue being heavily investigated by researchers, in order to establish an effective therapeutic protocol, which can alleviate both the source and the symptoms of the disease. The annual expenditures of this disease keep rising without significant progress in treating the pathogenesis of the disease (Thies et al., 2013). It seems that the directions of research should point more towards attacking the epigenetic deregulation causing the disease rather than allowing the major focus to be the treatment of symptoms. Overwhelming evidence has been presented in the recent years that show potential avenues of epigenetic treatments, and these avenues should be strongly pursued in order to make tremendous advances in the management of this disease.

With further research into targeted inhibition of each class of HDACs, ways to augment methylation status in the AD brain, and specific up/down regulation of miRNA, strides can be taken into discovering a cure for this expensive and consuming disorder. Although healthy lifestyles do help prevent the progression of AD in a predisposed individual, a cure needs to be found because of the genetic backing this disease has. Slowing down the environmental component of AD and treating the symptoms of the disease are both useful methods of management, but they are by no means sufficient in treating and curing the disease. The most promising outlook into curing the disease in our current state of knowledge of AD is through manipulation of epigenetic regulation.
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Volunteer Experience:

The following volunteer work was done at the Florida Hospital – Orlando: from August 2010 – September 2011.

Patient Companion Volunteer:

• Assisted in increasing the comfort of patients during their stay in the hospital by giving them attention and showing them much needed love while their families were away.
• Received training on acceptable bedside manner with patients.
• Interacted with various patients, of all temperaments, and applied my training to facilitate smooth interactions.

Pediatrics Volunteer:

• Was an experienced member of the pediatrics volunteers; I trained new members on occasion.
• Assisted nurses and child-life specialists in the pediatrics department in anything they required.
• Applied proper cleaning protocol to clean items handled by patients.
• Learned the subtleties of interacting with children (ensuring that they have fun, but also that they don’t get out of control.)
• Handled and fed infants on a daily basis.
• In charge of pet therapy for 1 month
  - Different animals were brought directly to patient rooms so that the children could interact with them and enjoy the animals company.

Research Experience:

Florida Hospital Neuroscience Clinical Research Institute: August 2010 – August 2011

- Research Assistant for the Florida Hospital Neuroscience and Orthopaedics department; approximately 8 hrs per week.
- CITI and IRB certified.
- National Institute of Health Stroke Scale (NIHSS) certified.
- Actively participating in Dr. St. Louis’s Normal Pressure Hydrocephalus study under the direct mentorship of Dr. Bright Wong, Neuroradiologist.
  • Examine and extrapolate data from patient brain scans (CT, MRI, X-Ray, PET)
  • Received training to measure Corpus Callosal/ACPC ratio and the Evan’s Ratio.
  • Assisting Dr. Wong in drafting the case study for this project.
- Constructed a Case Report Form (CRF) for Dr. St. Louis’s study pertaining to various spinal surgeries. This form was used to compile patient demographics, surgery-specific conditions, etc.
- Collected data from electronic medical records for Dr. Ademola Adewale’s study regarding brain tumors.
- Collected data from electronic medical records for Dr. Ademola Adewale’s demographic analysis of hemorrhagic strokes.