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Investigating Neurogenesis as a Veritable Epigenetic Endophenotype for Alzheimer's Disease

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Investigating Neurogenesis as a Veritable Epigenetic Endophenotype for Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease, characterized by progressive amyloid plaque aggregation, neurofibrillary tangles, and cortical tissue death. As the prevalence of AD is projected to climb in coming years, there is a vested interest in identifying endophenotypes by which to improve diagnostics and direct clinical interventions. The risk for complex disorders, such as AD, is influenced by multiple genetic, environmental, and lifestyle factors. Significant strides have been made in identifying genetic variants linked to AD through the genome-wide association study (GWAS). It has been estimated in more recent years, however, that GWAS-identified variants account for limited AD heritability, suggesting the role of non-sequence genetic mechanisms, such as epigenetic moderators. By influencing gene expression, epigenetic markers have been linked to age-associated decline through modulation of chromatin architecture and global genome instability, though such mechanisms are also involved with a number of normal biological processes, including neurogenesis. As the strategies of clinical genetics shift to include a heavier focus on epigenetic contributors, altered adult neurogenesis presents itself as a strong candidate for an endophenotype of AD development. This thesis proposes that, due to neuropathological dysfunction of epigenetic mechanisms in AD, new generations of neurons fail to proliferate, differentiate, and mature correctly, resulting in the larger loss of neurons and cognitive deficits characteristic to neurodegenerative disease. The plasticity of the epigenome and the role of epigenetic factors as mediators of the genome and the environment make such alterations attractive in AD research and implies the potential for therapeutic interventions. The present review submits neurogenesis as a viable target of epigenetic research in AD, highlights shared loci between neurogenesis and AD in the epigenome, and considers the promises and limitations of the neurogenic endophenotype.

Section I: Introduction

Advancements in technology and healthcare access in the last several decades have resulted in a continuous increase in life expectancy. Population projections in the United States show that the number of elderly Americans will rise dramatically through 2050. This steady march of America's baby boomers into old age corresponds with an increasing prevalence of Alzheimer's disease (AD) and other neurodegenerative disorders and their accompanying need for the allocation of resources for its patients' care and treatment. Research by Bredfeldt et al. (2015) examines the current trajectory and economic impact of AD, concluding that the prevalence and incidence of the disorder, as well as its long-term care spending costs, pose a significant obligation to the economy. Their research estimates that, as the first baby boomers reach their 60s and early 70s, the prevalence of AD in the United States will be 1.2% in 2020 (Bredfeldt et al., 2015); current estimates suggest that 5.7 million Americans have AD, indicating a national prevalence of 1.75%, already higher than previous projections for the year 2020. Predictions from Cornuitiu (2015) corroborate these findings, adding that, by the year 2040, more than twice as many baby boomers will have AD (10.3 million) compared to those of their equivalent ages in 2015 (4.7 million) (Cornuitiu, 2015). These results are especially disquieting when it must be acknowledged that AD, dementia, and other age-moderated cognitive impairments are not synonymous. Rather, the burden of these non-AD disorders is not covered by these estimates, and, therefore, understates the population that will be affected by neurodegeneration in the coming years.

Neurodegenerative disorders are characterized by progressive neuronal death and cellular dysfunction; as such, aging constitutes an important risk factor. The most endemic of these disorders, including AD, Parkinson's disease (PD), frontotemporal lobar degeneration

(FTL/D), and amyotrophic lateral sclerosis (ALS), have no effective treatments (Harari & Cruchaga, 2016). Of the many varieties of neurodegenerative disorders (NDD), marked cell loss is a trait shared by all, though many also exhibit characteristics of abnormal and dysfunctional axons, neurites, and a decline in the neurotransmitter network both before and during neuronal loss. Investigative studies into the causes and underlying mechanisms to complex neurological disorders are still in their infancy. Barring no treatments to delay onset or provide a cure, estimates on the incidence of AD and other NDDs are expected to reach epidemic proportions within the foreseeable future (Allen et al., 2014). Geriatric populations are already immensely affected by the disease, with nearly one in nine Americans aged 65 and older has AD; however, by the time this population reaches age 85 and older, one in three (32%) will have AD (Allen et al., 2014). It is the sixth-leading cause of death in the United States, and the fifth-leading cause of death among those ages 65 and older, behind heart disease, cancer, chronic obstructive pulmonary disease (COPD), and cerebrovascular disease or stroke (Hsu & Marshall, 2018). As a cause of death, AD increased significantly from 1979 to 1988, where it stabilized before gradually increasing again starting in 1992. Presently, AD is the most common type of age-related neurodegenerative disorder (Allen et al., 2014). Like many disorders with a neurological component, AD may have experienced this increase due to improvements in post-mortem diagnosis, facilitation in reporting, and a wider knowledge of the condition within medical communities (Hoyert, 1996).

According to Allen et al. (2014), there were five FDA-approved drugs for providing temporary relief of symptoms in some patients later diagnosed with AD. None of these therapies slowed or halted disease progression, however. In addition, several non-pharmacological therapies have been released, aimed at improving quality of life through

symptom management. These tactics include physical therapy, reminiscence therapy, and cognitive stimulations (Allen et al., 2014). The rising epidemic possibility and treatment futility demonstrate the importance of apprehending neurodegeneration in the coming years. The National Institute on Aging proposes that precision medicine may be the key to finding the most effective ways to diagnose, treat, and prevent diseases, such as AD, in an individual. This approach takes into account personal variability in genes, environment, and lifestyle to create a more accurate risk assessment and targeted treatment plans for diverse populations.

Multifactorial genetic disorders, such as AD, are likely influenced by multiple genetic and environmental factors. Critical developments in recent research suggests that some of these factors influence disease risk through effects on gene expression (Allen et al., 2014). Rising in popularity and feasibility within complex disease research is the prospect of epigenetics. Epigenetics is a field of study focused on heritable changes in gene expression that do not implicate alterations to an individual's underlying DNA sequence. These mechanisms translate external experiences into neural signals which launch the production of gene regulatory elements inside a cell. Gene regulatory proteins attract or repel enzymes that add or remove epigenetic markers. These markers control protein production by activating or repressing genes, thereby shaping how organisms function. These changes occur throughout normal growth and development, bearing crucial involvement in cellular biodiversity and differentiation; however, they are also linked to more damaging processes, such as tumor formation. While much of the original investment in epigenetics lay in genetic assimilation and an organism's stress response (Waddington, 1942), there has been a renewed interest in the reversible properties of epigenetic changes and their clinical implications in cancer, immune disorders, and neuropsychiatric pathologies.

Many notably heritable diseases, including AD, are moderated by complex interactions of genetic and environmental factors which shape an individual's risk susceptibility. Such environmental factors include diet, physical exercise, exposure to toxic substances, and viruses, which can not only predispose an individual to a given disorder but also, if left untreated or unaltered, can induce a chronic inflammatory response (Grant et al., 2002). Exposure to toxins, such as heavy metals, has been linked to an increase in oxidative stress in neurons and subsequent cell death and neurodegeneration. While the impact of the environment on the pathological pathways of disease cannot be ignored in complex diseases, the interaction of well-replicated susceptibility genes and environmental factors may provide more empirical information on disease development. There are a great deal of studies, including many which precede the recent revolution of genomic research; they outline an etiological perspective on disease risk and progression, highlighting the role of the environment. These publications interrogate particular risk and protective factors across populations and geographic regions, through occupational and ecological studies. The purpose of this research, however is to recognize the underlying susceptibility networks which predispose an individual to AD and the ways in which developing epigenetic research can inform an improved endophenotype for this neurodegenerative disease.

While epigenetic research grows, so, too, does the urgency for a reliable endophenotype by which to diagnose and treat the complex disorders these changes moderate. Post mortem hallmarks are unaccommodating to diseases which progress *in* vivo, and their use poorly reflects the innovation and development of newer imaging and sequencing technologies. As a result, neuroscientists and clinicians alike seek a new biomarker for AD, one which might be detectable prior to a patient's death and, ideally,

whose development begins prior to cognitive deficits. In recent years, neurogenesis has become a popular candidate for this endophenotype (Horgusluoglu et al., 2016), as its dysfunction within the adult brain would reflect a considerable overlap with the cognitive deficits linked to neurodegenerative disorders. During development, the structure of the nervous system is established through the precise and ordered production of neurons. In many regions of the brain, new neurons do not form after early development; however, in the dentate gyrus and in the olfactory bulb, the production of new neurons, or neurogenesis, occurs throughout life (Lledo et al., 2006), though the causes are not entirely clear. Neurodegeneration was described previously as a process of orchestrated cell death in a disease case. To fully encapsulate neurodegenerative disorders, however, it must be considered that neurons are not only dying at abnormal levels but that they also may not be produced correctly or may not migrate at the same quality and pace of non-diseased neurons. In recent years, neurogenesis has asserted itself within a growing clinical interest (Fig. 1). Publication data from the last several decades demonstrates the immense attention paid to AD and epigenetics and the early signs of neurogenesis invocation in these journals.

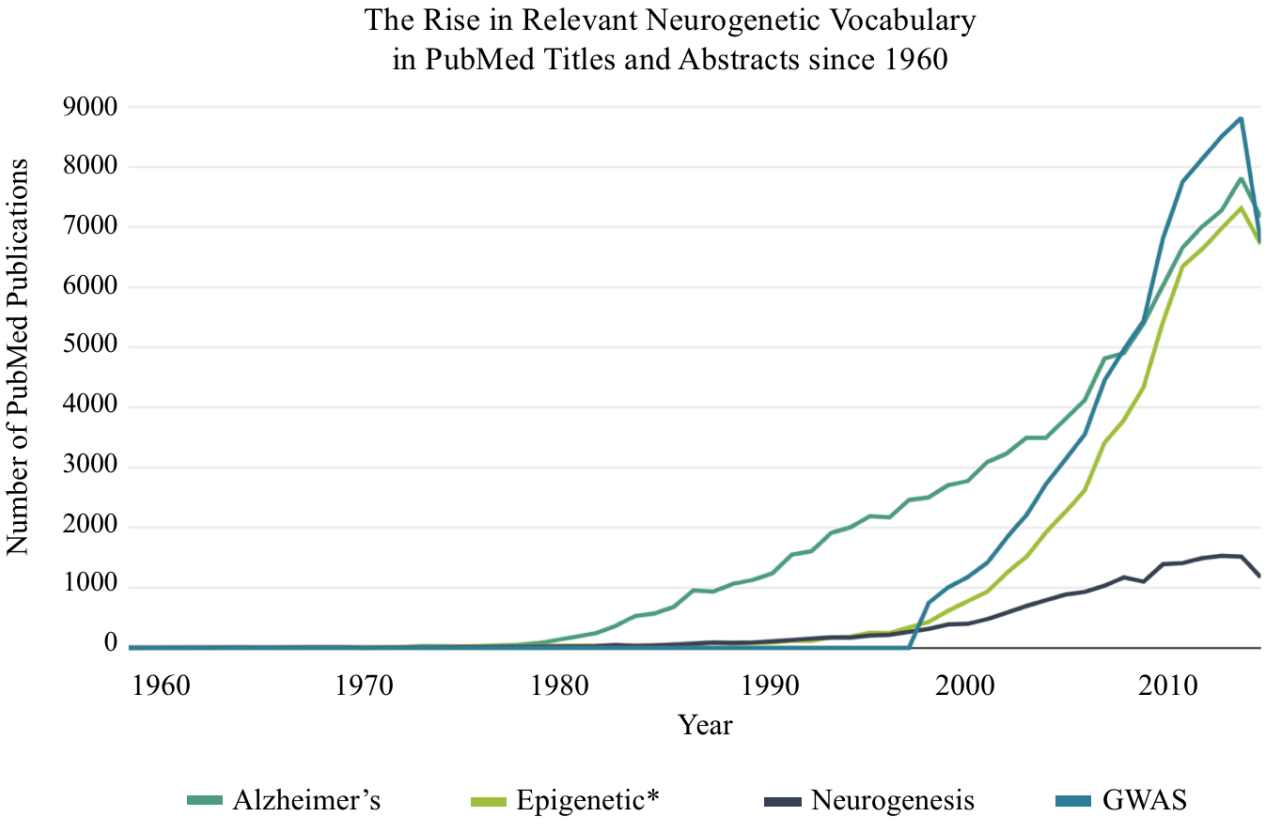

Figure 1. The increase in usage for "Alzheimer's," "Epigenetic*," "Neurogenesis," and "GWAS" in PubMed publication titles and abstracts since 1960, using data compiled on October 1, 2018. This data uses the number of publications as a proxy for growing scientific investment in the neurogenetic field and applies it to Alzheimer's disease.

Recent publications have shown a vested interest in AD and a growing presence of epigenetics in clinical research. The integration of epigenetic perspectives in understanding AD is reflected in the growing overlap between "Alzheimer's" and "Epigenetic*" in PubMed publications (Fig. 2). As imaging technologies and sequencing arrays improve, neurogenesis continues to gain traction within larger spheres; for example, immunohistochemistry within human brain tissue samples has been used to link neurogenic processes with pre-existing neural networks (Li et al., 2008).

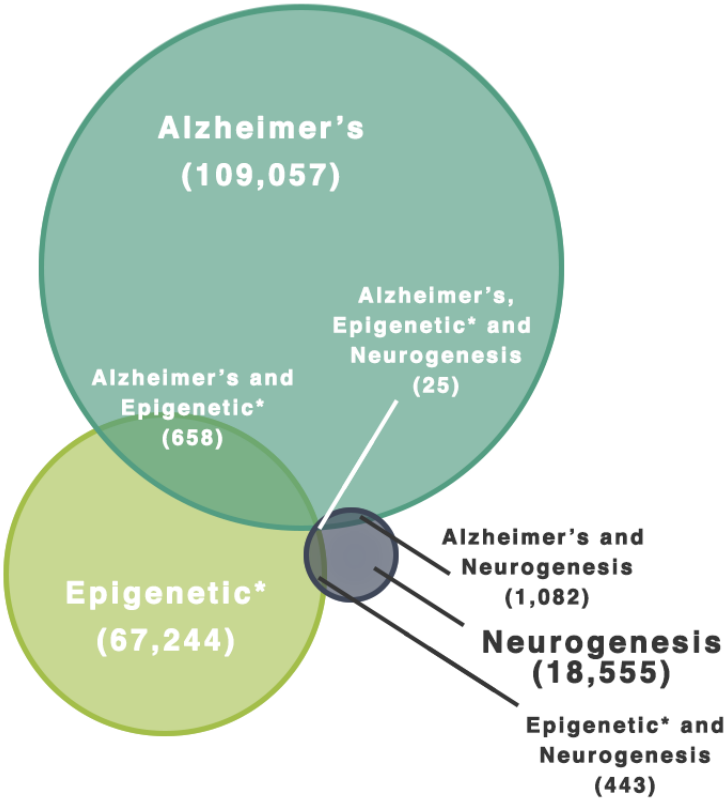

Figure 2. The intersection of "Epigenetic*", "Neurogenesis", and "Alzheimer's" in number hits within titles and abstracts of PubMed articles, using data compiled on October 1, 2018. These publications advance knowledge and strategies which promise to yield a more interdisciplinary approach to understanding diseases.

This thesis seeks to steer the course of genetic and epigenetic research into the etiology of AD towards neurogenesis, a still not entirely understood biological process of cell growth and development in the nervous system. It aims to answer the questions as to whether AD and other neurodegenerative disorders are a product of cell death alone, or, as more recent research would suggest, the result of neuron death as well as a dysfunction in replacing and regenerating synapses. Recent research has indicated that neurogenic processes are under extensive epigenetic control and are subject to alteration through environmental factors, such as physical exercise and enriched environment (Yao & Jin, 2014). Furthermore, in the years to come, it will be imperative to understand and distinguish healthy from disordered aging, especially as it pertains to cognitive decline and deficits in learning and memory. Through an analysis of these increasingly intersecting fields, I hope to contribute to

a newer strategy of unraveling complex disease etiology by looking for patterns of gene expression and relating them to patterns of the neural landscape seen in disease cases. The coming years represent an important inflection point within neurogenetics, in which the field of epigenetics must turn a corner to best address the growing incidence of AD.

Section II: The Model System for Neuropsychiatric Genetic Research

Though it has been over a century since the first description of AD, in a 1906 publication by Alois Alzheimer, the German psychiatrist and pathologist who would lend the disease his name, our capacity for diagnosing and treating the disease has improved little. The Alzheimer's Association identifies three general stages of AD progression: mild AD, or early stage; moderate AD, or middle stage; and severe AD, or late stage. Early symptoms include a decreased ability to focus and reason and a loss of memory, but later stages of the disease are characterized by greater cognitive decline, mood instabilities, and abnormalities in coordinated movement (Donovan et al., 2014). Neurological changes related to the progression of AD begin years before symptoms of the disease can be recognized; this period of preclinical development can last for years. An individual's risk of acquiring AD is influenced by complex interactions between genetic risk susceptibility, epigenetic modifications, and environmental risk factors (Gangisetty, 2018).

Two major hallmarks of AD pathology that have arisen in the last decade are the accumulation of amyloid-β plaques and tangles of intracellular hyperphosphorylated tau protein. It is thought that the abnormal cleavage of the amyloid precursor protein (APP) leads to an excess of amyloid-β peptide accumulation, in accordance with the "amyloid cascade" hypothesis (Hardy & Higgins, 1992). These peptides form oligomer aggregates within extracellular neuronal synapses. This protein is not unique to humans, but, rather, it is largely conserved throughout many species, from Drosophila to humans (Cassar & Kretzschmar, 2016). However, this specific cleavage of APP produces toxic intermediate protein aggregates, containing an insoluble form of amyloid-β (Allen et al., 2014; Gangisetty, 2018). Amyloid-β has been linked to mitochondrial dysfunction, inflammation, and synaptotoxicity

(Selko, 2002). When tau, a microtubule-associated protein (MAP), becomes hyperphosphorylated in neurons, it prefers itself to the cytoskeletal element. The result is an intraneuronal neurofibrillary tangle (Gangisetty, 2018; Selko, 2011) (Fig. 3). These structures are also present in other neurodegenerative diseases, such as Parkinson's disease, where they are known as tauopathies.

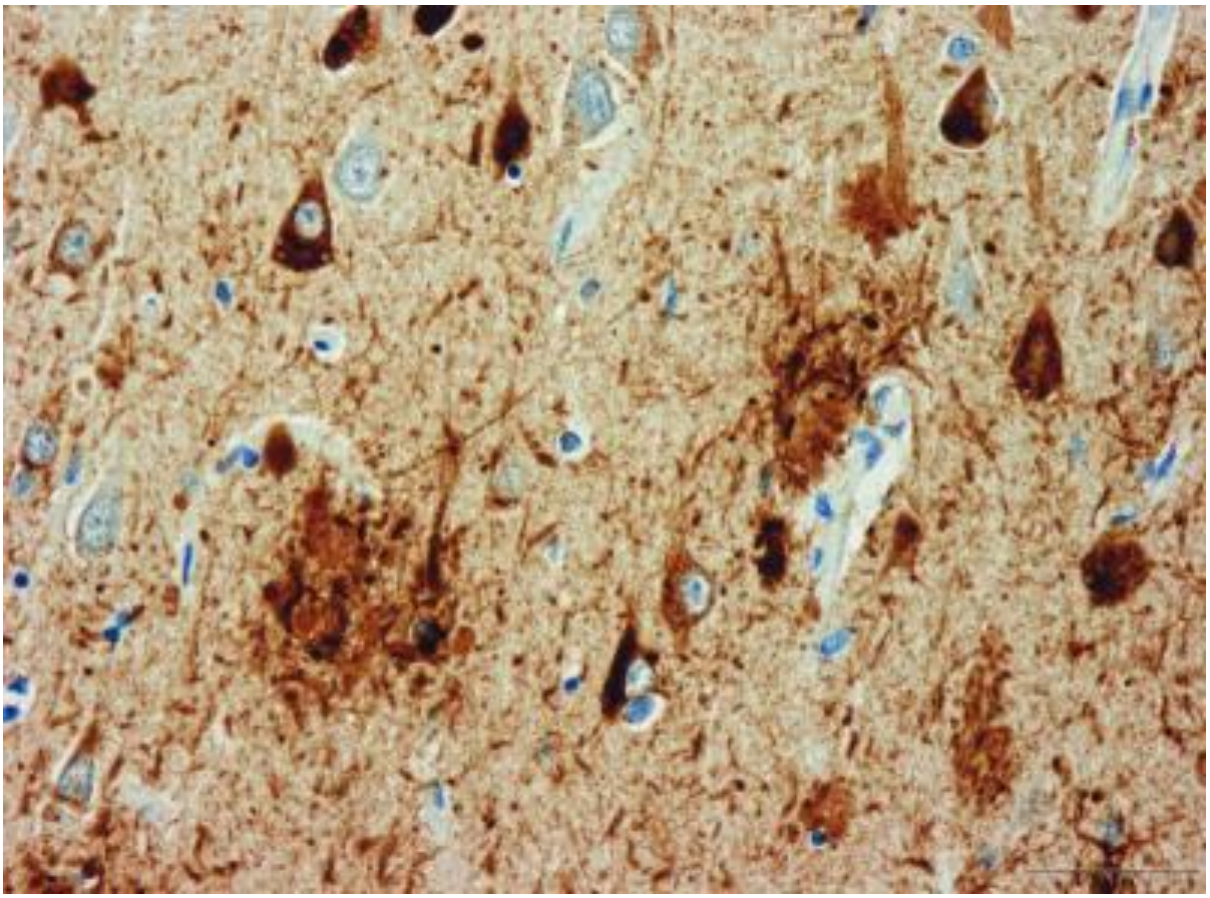

Figure 3. Human tissue from the hippocampal region of the brain. Intracellular tau protein tangles are stained brown, forming triangular shapes in excitatory neurons. Amyloid-β proteins comprise the sparse, round structures within the extracellular matrix. Credit: Washington University School of Medicine (Kauwe et al., 2008)

Aside from protein aggregates, neuroscientists have also characterized AD as a disorder of neuronal loss. With disease progression comes a loss of connectivity between

neurons and their subsequent atrophy of brain tissue and cell death. These biological markers are well-recognized and reliably-characterized in post-mortem brain samples. However, little is still known about the disease's underlying risk factors and the molecular mechanisms implicated in disease progression (Freytag et al., 2018). The previously mentioned biomarkers, or, more accurately, necro-biomarkers, reflect a considerable challenge in AD research; a definitive diagnosis of AD can only be made during a neuropathological examination during autopsy, due to the relative inaccessibility of cortical tissue and the lack of reliable biomarker for complex pathologies. Therefore, there is a vested interest in the diagnostic and prognostic applications of a reliable clinical endophenotype for this disorder.

There are two types of AD recognized by the National Institute on Aging: familial and sporadic, characterized by early- and late- onset, respectively. Familial Alzheimer's Disease, of the early-onset variety, only accounts for 5% of AD cases. It is caused by discrete genetic mutations, passed through families. In these families, symptoms typically present well before the age of 65, sometimes as early as 30 or 40 (Donovan et al., 2014). Three genes have been associated with Early-Onset Familial Alzheimer's Disease (EOFAD), and mutations in these genes follow a pattern of autosomal dominant inheritance. They are the amyloid precursor protein (APP) on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, and presenilin 2 (PSEN2) on chromosome 1. Each presenilin gene encodes an enzymatic unit involved in the metabolism of APP; mutations of these genes can result in the specific cleavage of APP which results in Aβ, the toxic specie. These extracellular proteins aggregate to form dense plaques. The predictability of EOFAD and its pathogenic loci has created a platform for the critical research into other variations of AD, including the more-common late-onset form. By observing AD-related neurological changes that occur in these families

before cognition deficits appear, researcher hope to link the formation of brain abnormalities with disease development and its underlying mechanisms that can be applied to the more common form of AD.

The more common form of AD, Late Onset Alzheimer's disease, is characterized by symptom manifestation in the mid-60s and later. While its cause and mechanisms are not yet completely understood, they are likely to include a combination of a complex genetic architecture and environmental and lifestyle risk factors. In the last five years, however, one reliable candidate gene has been found to moderate AD risk. The apolipoprotein (APOE) gene, located on chromosome 19, presents in three alleles: ε2, ε3, and ε4. APOE ε2 is a relatively rare allele which protects against disease risk. It is thought that this allele delays the onset of AD in these individuals, compared to APOE ε3 or APOE ε4 variants (Liu et al., 2013). APOE ε3 is the most common allele for this gene. It is believed to play a neutral role in the disease, neither increasing nor decreasing risk of acquisition. The APOE ε4 allele, however, substantially increases risk for AD and has been linked to an earlier age of onset. The number of APOE variant alleles in an individual's genotype significantly alters their susceptibility to AD (see Appendix A). About 25% of the population is heterozygous for APOE ε4, corresponding to a three-fold increased risk for AD. An individual who is homozygous for APOE ε4, which occurs in approximately 1% of the population, has a ten- to 12-fold increased risk of developing AD (Verghese et al., 2011). While a blood test can identify which APOE alleles a person has, these results are unreliable for predicting who will or will not develop the disease and to what severity.

As a heterogeneous disease attributed to a vast number of combining genetic and environmental factors, the most important and most reliable risk factor for AD is age (Ridge

et al., 2013; Weill Institute for Neurosciences, 2018). Specifically, it appears that AD is not simply an accelerated progression of the normal aging process, but, rather, a systemic pattern of dysregulated aging; genetic research speculates that this dysregulation may induce disordered changes to the structure of chromatin, the condensed configuration of DNA. In this disease, genetic factors do not act alone and are joined by a host of potentially harmful environmental and lifestyle influences. Environmental and behavioral risk factors include hypertension, estrogen supplements, smoking, stroke, heart disease, depression, arthritis, and diabetes (Cornuitiu, 2015). Additionally, some lifestyle choices appear to decrease the risk of AD, including exercise, intellectual stimulation, and maintaining a Mediterranean or pescatarian diet (Ridge et al., 2013). Contemporary research seeks to link these external factors to biological mechanisms for a more comprehensive understanding of disease risk acquisition and protective elements.

Research from Lunnon et al. (2014) outlines two specific reasons as to why cause-and-effect research is particularly challenging for AD. First, brain tissues are uniquely inaccessible, and, second, this age-related disorder is poorly suited for longitudinal study. By the time AD-related cognitive deficits manifest, there are a number of age-confounding variables which only increase, further complicating symptomatologic observations. Furthermore, the etiological process of AD remains unknown, resulting in an unclear timeline of symptom manifestation and underlying biological dysfunction. In order to best model AD and other complex disorders, two main models have been proposed for its study.

> "Two seemingly contradictory hypotheses exist about the architecture of complex disease: the common disease/common variant hypothesis and the multiple rare variant hypothesis. In the first, many common variants of small effect size collectively explain disease risk, while in the second, rare variants, some with large effect and high penetrance, explain disease risk." (Ridge et al., 2013; Singleton & Hardy, 2011).

Singleton & Hardy (2011) suggest that these hypotheses may not be mutually exclusive; the genetic landscape governing complex diseases, such as AD, is likely a hybrid of the two possibilities. They suggest that both common and rare variants are efficacious in increasing or decreasing disease risk, and, very often, they are found in the same loci, called, "pleomorphic risk loci" (Ridge et al., 2013; Singleton & Hardy, 2011). The ability for research teams of today to model genetic variants across a prevalence and effect size matrix provides a blueprint for the design and development of efficacious therapeutic interventions. As a complex disorder with genetic influences and a great investment into its treatment, AD poses an attractive disease by which to apply a pleomorphic risk strategy.

The co-occurrence of AD large body of research, epidemic possibility, steadfast financial and scientific investment in the search for answers in heritability and causation, and the current limitations of traditional brain imaging research methods make the disorder the prime candidate for utilizing the newer, promising modes of genetic research. The advent of more complex, high throughput next generation sequencing and the falling costs of this technology make this an ideal time to expand AD research into previously unexplored territory, such as seeking out rare variants or continuing the search for a reliable genetic biomarker. As a model system, it is hoped that the devotion to comprehending AD will yield clues to understanding other neurodegenerative disorders for which there is not yet such a large literature base.

Section III. Uncovering the Missing Heritability of Alzheimer's Disease

Heritability is defined as the proportion of variance in a disease that can be explained by genetic variation. More precisely, heritability is the proportion of variance due to the effects of additive genetic components, including allelic variants, epistatic interactions, or DNA sequence variants. As it pertains to human disorders, a seminal work on inheritance explains that "the variability of presentation of...complex diseases has a component of quantitative inheritance, consisting of the effects of different allelic forms that interact with each other and with the environment" (Blanco-Gomez et al., 2016). In an effort to uncover the genetic architecture underlying such common, complex disorders, as well as rare Mendelian diseases, research teams of the early 2000s relied on the momentum and data stemming from the Human Genome Project. Their goal was to determine genetic risk factors for diseases and use them to make predictions about the dysfunction and biological mechanisms underlying an individual's risk.

The culmination of these efforts was the birth of the genome-wide association study (GWAS), which produces replicable DNA sequence variations for a given phenotype. Using microarrays containing millions of single nucleotide polymorphisms (SNPs), collaborative GWASs have identified variants linked to complex diseases and traits (Lord & Cruchaga, 2014; Tak & Farnham, 2015) (see Appendix A). In a clinical case, GWASs rely on differences in the frequency of a specific SNP in healthy (or control) vs. diseased (or case) populations. When a SNP is identified by GWAS to be statistically significantly overrepresented in a disease population, it is called a risk-associated SNP; the surrounding genetic regions containing such SNPs are called risk loci for that particular disease. These studies into genetic variation help in identifying possible regions of the genome that are

implicated in the onset and progression of a disease. Advancements in the accuracy, speed, and cost of genotyping and its complementary analytical software have resulted in the identification of well-validated genetic candidates of risk for common disorders, such as arterial disease or type 2 diabetes (Freytag et al., 2018). Recognizing these genetic risk loci and understanding their underlying molecular mechanisms and functional relevance are two different things, however.

It is difficult to understand disease risk from GWAS results for a number of reasons, including individual SNP power and its location within the genome. Not all SNPs incur the same amount of risk for a disease, and, similarly, the presentation of SNPs in a genome does not suggest additive risk accumulation. Most GWAS-identified SNPs, either directly genotyped or imputed, are located in non-coding regions of the genome, presenting a puzzle as to how a single-nucleotide change in such regions can confer conditional risk of a disease (Tak & Farnham, 2015). In fact, some estimates indicate that most disease-associated index SNPs, about 88%, are located in non-coding regions of the genome, almost equally divided between intergenic (43%) and intronic (45%) regions (Blanco-Gomez, 2016; Tak & Farnham, 2015). To reconcile the influence of intronic variation, a current hypothesis presents the possibility that individual SNPs induce changes in gene expression levels as a post-translational modification, rather than the direct protein creation and function that might be seen from exonic variation. Because of this, it is critical moving forward to identify both the direct target of a risk-associated element and other genes affected by a change in expression levels of these direct targets, thereby integrating traditional genomic approaches with evolving expression-based approaches.

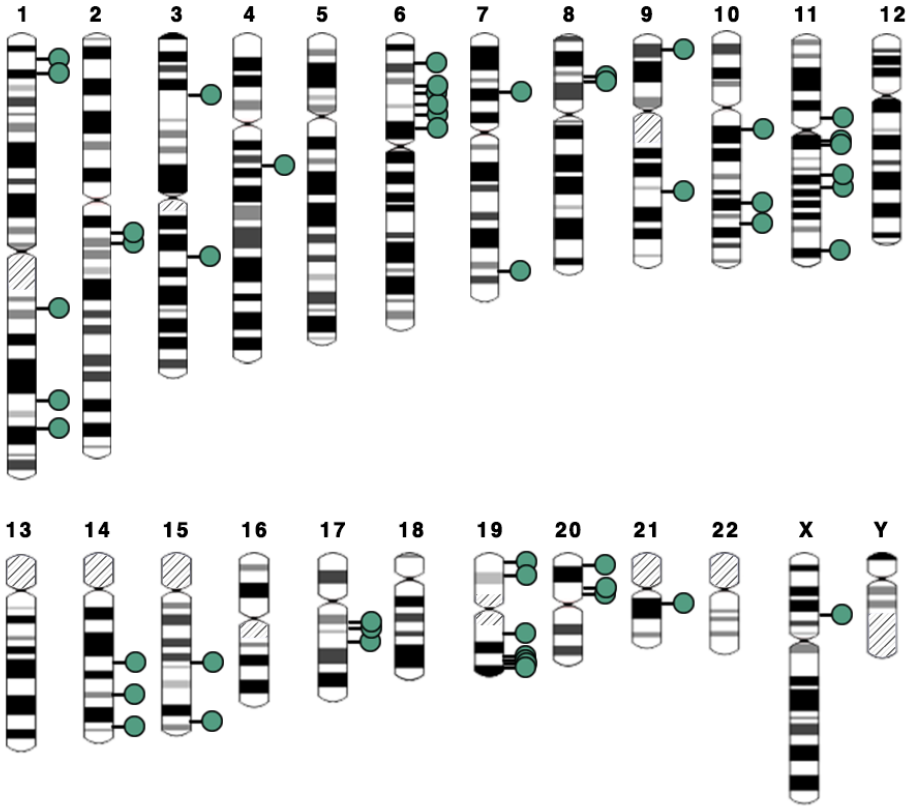

Figure 4. A chromosomal ideogram displaying the 50 most significant loci for AD uncovered by GWAS, using the genetic risk score algorithm designed by Chauhan et al. (2015). Teal dots represent individual loci. Specific gene names, locations, and functional relevance can be found in Appendix B.

Since their inception in 2002, GWAS have been very successful in identifying broader genetic regions associated with complex traits. Using these results, neuroscientists and geneticists have identified a number of candidate regions of interest along the genome that may contribute to an increased risk for late-onset AD (Donovan et al., 2014; Harari & Cruchaga, 2016) (Fig. 4). These regions, scattered across the genome, include genes which encode conformational changes in proteins and moderate inflammatory response, enzyme activity, and more. Each genetic loci represents a region which, when altered in an individual, manipulates their susceptibility for AD, based solely on genetic sequence. By design, GWAS studies are aimed at identifying common variants, and, as such, they are inconclusive in explaining the full heritability to intricate, multifactorial phenotypes, such as AD. Although many large GWAS publications on AD have been performed and replicated, a major part of the genetic component to phenotypic variability and susceptibility still has not

been found, a predicament known as "missing heritability," (Blanco-Gomez et al., 2016). In a meta-analysis of twin studies, it had been proposed that up to 80% of risk for Late-Onset AD is predicted to be accounted for by genetic influences, though the aforementioned variants account for less than 40% of the genetic component for AD (Allen et al., 2014).

Two research teams in the last five years have sought to uncover the magnitude of this missing heritability. A meta-analysis conducted by Lee et al. (2011), observing 3,333 cases and 3,924 controls, including 2,699 population-based estimated that common genetic variants, such as those discoverable in a GWAS, account for 24% of variance in AD. The same analysis gauged the contribution of the APOE gene, using several proxy SNPs with varying degrees of linkage disequilibrium; their estimate of the APOE effect was approximately 4%, though their review considered only directly genotyped SNPs (Lee et al., 2011). An analysis published by Ridge et al. (2013) reviewed both genotyped and HapMap imputed SNPs (see Appendix A). Using the Alzheimer's Disease Genetics Consortium (ADGC) dataset described by Naj et al. (2011), Ridge et al. (2013) note that, across all 2,042,116 SNPs imputed in the HapMap, 33.1% of phenotypic variance is explained. From the same data set, they note that the APOE ε2 and ε4 alleles, which are those that contribute to AD risk, account for 5.9% of the phenotypic variance. These estimates are significantly less conservative than those determined by Lee et al.. Leveraging these analyses, it is estimated that the missing heritability for AD in the post-GWAS era is about 65%.

In a genome-wide distribution analysis, Ridge et al. (2013) surmised that chromosome 19 accounted for the highest proportion of phenotypic variance. After accounting for the 11 most significant pre-established AD loci, this variance, when assigned across somatic chromosomes, denotes chromosomes 1, 4, 5, and 17 as those accounting for

the largest percentages of unexplained phenotypic variance (Fig. 5B). They found that each of those chromosomes accounts for more than 2% of variance while chromosomes 9, 14, and 21 account for the least variance, at roughly 0.0001% each (Ridge et al., 2013). Considering what has already been established regarding the influence of the APP gene on chromosome 21 and PSEN1 on chromosome 14 in EOFAD development and their functional relevance to the production of amyloid-β aggregations, this research suggests that there are other significant regions of the genome that demand attention in uncovering moderators of AD risk.

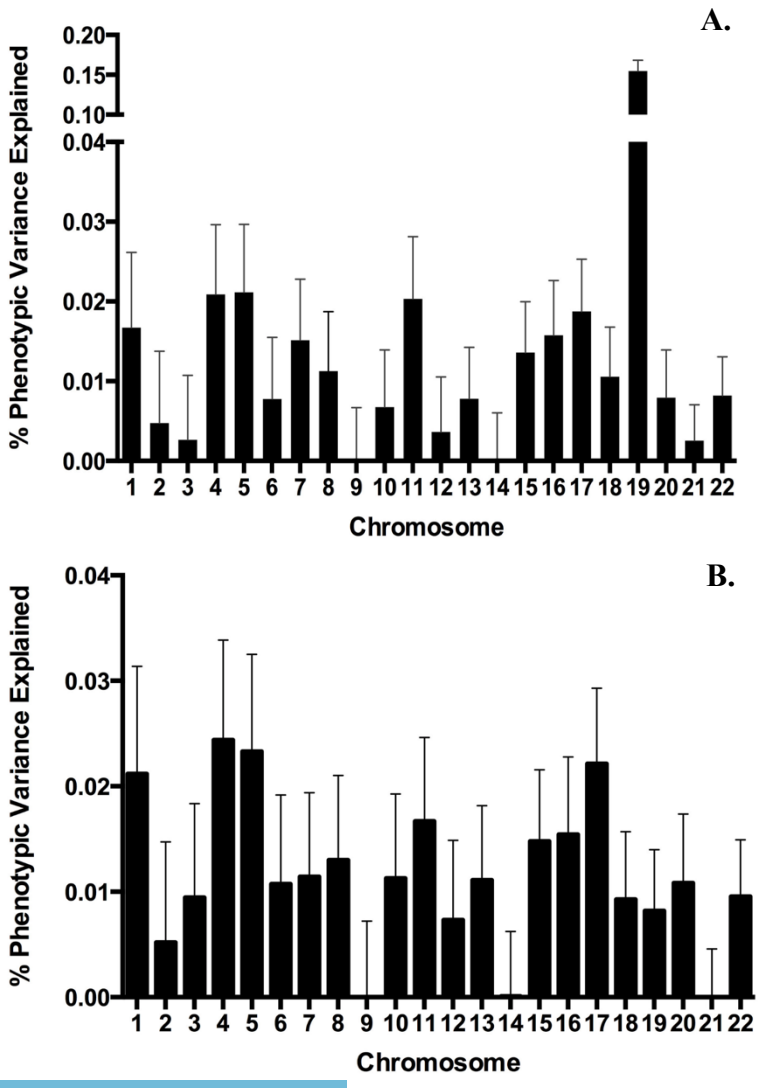

Figure 5. A. Unexplained AD variance, by chromosome. In this figure, Ridge et al. (2013) show phenotypic variance explained by all SNPs. Error bars correspond to standard error (Ridge et al., 2013, Fig. 1). B. Unexplained AD variance, by chromosome, excluding known AD markers. After accounting for the most significant AD-associated SNPs, explained phenotypic variance shifts to highlight other chromosomes (Ridget et al., 2013, Fig. 2).

Though the establishment of relevant AD loci has become tremendously thorough and targeted, GWAS research is reaching a limit to the amount of heritability it can explain. The circumscription of GWAS has ushered in a new generation of research, one which seeks to explore the epigenome and enrich our understanding of complex disease etiology and pathogenesis through non-sequence alterations (Klein et al., 2016). A common complication to genetic research is interpreting the biological distance between a genetic polymorphism and its consequences in a tissue of interest. Publications by Freytag et al. (2018) propose that this gap may be reduced through the interrogation of molecular mediators, such as gene expression. AD investigations have been at the forefront of this discipline, due to its global health interest, substantial funding, and previous body of research; in fact, it was a study into AD which produced the first independently replicated associations of an epigenomic marker for a disease (Klein et al., 2016).

This inflection point to the field is hinged on the implication of new areas of the genome involved in disease, including locating epigenetic targets for these traits. In the neuropsychiatric sphere, DNA methylation and chromatin structure of human brain tissue are proving pivotal points for facilitating ongoing genetic research (Klein et al., 2016). In their epigenetic review of AD, Freytag et al. (2018) write that "the integration of transcriptomics data in the study of the genetic factors of complex traits has significantly improved our understanding of their genetic basis. Thus, methods that reduce the gap between genetic susceptibility [estimates] and their functional [consequences] are expected to increase our understanding of the genetic underpinnings of genetically complex traits." With advancing technology and dropping costs to epigenetic research, the field of clinical genetics is

expected to shift to include complementary EWAS investigations in tandem with GWAS results.

The epigenome offers an attractive solution to capture information not just about actively transcribed genes, but also those genes which have the potential to be expressed in the presence of a particular stimulus. The effect of the environment into cognitive principles, such as brain development, learning and memory, and other higher-level cognitive functions is one of the earliest and foremost implications of epigenetic mechanisms in neuroscience (Gangisetty, 2018). The following generation of epigenomic studies in Alzheimer's Disease seeks to isolate the effects of AD compared to standard effects of aging. Previous research had been difficult to interpret for a variety of reasons, including small sample sizes; the lack of accounting for confounding variables, such as age; and a lack of replication in independent samples (Klein et al., 2016). The sequential replication design across multiple tissues utilized by Lunnon et al. (2014) represents the first epigenome-wide association study (EWAS) of AD. This style of association study is motivated by an increasing knowledge of the architecture of the genome. In turn, the results of these studies better informs the epigenetic landscape of complex disorders and affirms the promising relevance of epigenetic variation in human health and disease.

Section IV. Alzheimer's Disease's Epigenetic Landscape

Understanding the epigenetic events underlying complex phenotypes is one of the first steps in increasing our knowledge of the biology of disease and constructing successful therapeutic efforts for historically untreatable conditions, like psychiatric and neurodegenerative disorders. Epigenetic changes are known to be implicated in a number of complex traits and syndromes, including some kinds of cancer and diabetes, Prader-Willi syndrome, and Angelman syndrome (Harari & Cruchaga, 2016). These mechanisms can alter gene expression with respect to the brain in memory formation and learning, two character hallmarks of dysfunction to AD. While genetic variation via SNPs or other sequence based variants are considered to be unchangeable, the epigenome is highly plastic. Epigenetic modifications exercise control through transcriptional activity and gene expression, making them reversible and susceptible to manipulation. Such markers respond uniquely to an individual's environment and life experiences and can precede disease pathology, indicating their potential as diagnostic tools or indicators of risk (Kelly et al., 2010). Each cell has its own distinct epigenome, featuring up to 40 different currently-defined epigenomic features, including DNA methylation, histone acetylation, chromatin alterations, X-inactivation, and imprinting. While this cellular heterogeneity makes tissue-level profiling a challenge in interpretation, it also contributes helpful information to the greater understanding of disease complexity. Furthermore, because epigenetic marks are hereditable and reversible, they have emerged as targets for clinical interventions and carrier research.

"Epigenetics" first made its way into empirical vocabulary in 1939, with geneticist Conrad H. Waddington's attempts to reconcile the old biological debate between epigenesis and preformationism. The term was proposed to describe the carefully-orchestrated

molecular events which take place in early embryonic development, using the Greek root for "over" or "above" to illustrate interacting mechanisms which give rise to variation in tissue and organ type not already present in a single gamete (Waddington, 1939). Today, epigenetics refers to a dynamic field which encompasses heritable changes in gene expression that do not involve changes to the underlying DNA sequence. As such, these changes can vary across time and tissue, unlike DNA, whose pattern is precisely replicated for all cells in a given individual, assuming no mutation. While some of these changes are inherited from parents, many, if not most, are acquired through environmental or lifestyle effects and can remain stable for long periods of time (Klein et al., 2016). The most common epigenetic events in relation to disease are DNA or chromatin alteration, as well as RNA-mediated modifications.

DNA Methylation

In part due to its relative ease of access in human brain tissue, most aging studies have focused on the epigenetic target of DNA methylation. Longitudinal surveys of samples from the human prefrontal cortex (PFC) have uncovered large differences in methylation over early development and aging. The latest advances in technology enable researchers to screen for methylated regions of the genome, using large numbers of samples in commercial genomic arrays. Methylation is, by definition, the addition of a methyl molecule in a CpG group of DNA; the downstream effects of methylation present a powerful mechanism for silencing gene expression (Harari & Cruchaga, 2016). The addition of methyl groups to DNA is a normal event that acts to stabilize the genome, as large quantities of DNA could otherwise interact in unpredictable recombination events or induce transcriptional dysfunction of nearby genes. The knowledge of these general patterns of methylation

establish a foundation upon which further studies may be conducted into the extent, mechanisms, and causes of interindividual DNA methylation variance and disease. Furthermore, there are a number of factors known to contribute to changes in methylation levels across the genome, including carcinogen exposure, such as tobacco, alcohol, arsenic, and asbestos; subjection to heavy metals; and diet. These environmental factors alongside the standard aging process have been hypothesized to influence clinically significant changes in the methylome.

The normal aging process induces a shift in the distribution of methylation across the genome. In older organisms, DNA hypomethylation results in a widespread reduction of inhibition across the genome, but, in some regions, DNA hypermethylation can also occur. In such cases of hypermethylation, which largely occur in promoter regions, many older patients show reduced gene expression (Gangisetty, 2018). Because the observed pattern of age-associated methylation is consistent in various tissue types, Christensen et al. (2009) suggest a common mechanism of dysregulation underlying the alterations. They propose a reduction in maintenance and precision of methyltransferases with aging in the case of hypomethylation or a potential accumulation of stochastic methylation events over time, in the case of hypermethylation. While the samples studied here do not present disease etiologies, the accumulation of epigenetic changes without detectable phenotypes cannot be written off as insignificant; furthermore such information can provide a baseline by which to distinguish and compare methylation levels between AD and older populations. It is possible, if not probable, that age-related drift without dramatic changes in gene expression may confer an increased susceptibility to disorders. The potential to increase pathological

risk can depend on the likelihood and frequency of methylation alterations, modifying overall genome stability.

Through three independent post-mortem cohorts, Lunnon et al. (2014) conducted a cross-tissue analysis of DNA methylation in AD. They identified a region within the ankyrin 1 (ANK1) gene that is differentially methylated from healthy controls. The ANK1 gene encodes a brain-expressed protein involved in the organization of neuronal plasma membranes (Lunnon et al., 2014); previous research associates the gene with neuropathology in the entorhinal cortex (EC). This gene was confirmed to be significantly hypermethylated in two other cortical regions: the superior temporal gyrus and the prefrontal cortex (Lunnon et al., 2014). The cerebellum, which is largely protected from the neurodegenerative processes in AD, did not show this pattern of hypermethylation. De Jager et al. (2014) confirmed 71 discrete CpGs corresponding to 60 differentially methylated regions, including two previously identified AD-associated loci identified by GWAS. Their results demonstrated that many of these differentially methylated positions were linked to genes within known AD susceptibility networks. These networks were derived from protein-protein interactions, rare variant studies, and GWAS candidate genes. This study presents one of the earliest robust association studies between AD and methylation patterns in brain regions known to be affected by the disease. It also establishes that simply averaging methylation measures over a gene is overly simplistic; the context of each CG-methylation is crucial to interpreting the effect size of an epigenetic change.

Histone Modification and Chromatin

Apart from DNA methylation, another group of prevalent, influential epigenetic marks is the class of histone modifications. This category refers to post-translational changes

that affect the wrapping and condensation of genetic material. DNA is wrapped around histone proteins to form nucleosome "beads on a string" (see Appendix A). This binding is one of the initial steps in condensing genetic material into the densely-packed chromatin. The normal biological aging process is characterized in part by a loss of heterochromatin, the compressed form DNA known to play a role in gene expression. This loss is demonstrated by DNA hypomethylation, the absence of heterochromatin marker H3K9me3, and selective dysfunction of the nuclear lamina (Klein et al., 2016). Histone modifications, such as acetylation, cause DNA to wrap more loosely around a histone protein, allowing for more gene activation via exposure. Acetylation is a dynamic and reversible process, regulated by histone deacetylases (HDACs) and histone acetyltransferases (HATs).

In the diseased aging process, covalent modifications begin to alter chromatin structure. For diseases associated with aging, such as AD, it would seem that histone modifications due to disease progression are joined by those induced in normal aging, producing a combined model of chromatin instability and additional malfunction in regulator binding, transcriptional initiation, and activity at enhancer regions (Gangisetty, 2018). In replicable animal models, global histone acetylation occurs in repetitive DNA elements in older mouse brains, which suggests a loss of chromatin integrity with age. Recent research has linked HDAC2 to the brain as an important regulator for synaptic plasticity and memory encoding, storage, and retrieval; an abundance of HDAC2 complexes resulted in increased synapse numbers and memory facilitation. Notably, this deacetylase has been shown to be disrupted in AD-associated processes, supporting the role of histone acetylation and deacetylation in AD (Guan et al., 2009; Li et al., 2008). The potency of histone modifications and loss of chromatin integrity with age or disease progression reinforce a

recurring theme using epigenetics to unearth larger biological and clinical implications in the future.

Research into the most common forms of histone modifications and their impact on AD pathology found that histone acetylation has an active role in disease susceptibility. Recent observations have tagged chromatin marker H4K16 for its implications in aging and neurodegeneration (Nativio et al., 2018). An analysis on the acetylation of H4K16 (H4K16ac) revealed a significant redistribution of marker between AD brains and matched age controls. Throughout the normal aging process, the amount of H4K16ac found across the epigenome increases to promote control over higher-order chromatin and regulate interactions at the level of the nucleosome. However, in AD brains, there is a marked loss of this epigenetic feature, particularly occurring near genes related to the AD and aging (Nativio et al., 2018). These findings suggest a model in which AD is more than an advanced state of the normal aging process; rather, the dysregulation of aging seen in AD pathology may induce structural and functional changes to genetic material, such as chromatin integrity. It also implies a mechanism by which health brains bear protective epigenetic marks that, when dysregulated, increase an individual's risk for disease development.

Non-coding RNAs

Since the transcription of non-coding RNAs (ncRNAs) is under direct epigenetic regulation, ncRNAs are implicated in a number of epigenetic processes, such as DNA methylation and histone modifications. ncRNAs are able to target enzymatic components of epigenetic machinery; as a result, they can directly influence the levels of RNA expression through other mechanisms, such as microRNA-related RNA degradation (Klein et al., 2016). Historically, most studies that have focused on small, non-coding RNAs revolved around

microRNAs, but the importance of long non-coding RNAs has become more evident in recent years. microRNAs (miRNAs) are small, no more than 20-24 nucleotide bases in length. They regulate gene expression post-transcriptionally by blocking translation or inducing the degradation of mRNA (Li et al., 2008). While many miRNAs are expressed throughout the human body, the brain shows an especially high presence of miRNAs. This suggests that miRNAs might play a role in neuronal development, function, and aging (Gangisetty, 2018). The role of miRNAs in AD has been observed in adult forebrains in knockout genetic studies. Using peripheral blood mononuclear cells, researchers found that, in an epigenomic profile of aging, the majority of miRNAs decrease with age. In particular, they found and validated nine different miRNAs that were significantly lower in older individuals compared to younger subjects, suggesting several regions of regulatory dysfunction with age (Noren Hooten et al., 2010).

Long non-coding RNAs (lncRNAs) are defined as heterogeneous regulatory elements greater than 200 nucleotides in length. At a lower level of function, lncRNAs are involved in post-transcriptional modifications, such as mRNA stability, splicing, and translation. Their higher-level applications are vast, spanning a number of biological processes, such as development, cell differentiation, cell survival, apoptosis, gene imprinting, and stem cell maintenance (Gangisetty, 2018). When lncRNAs couple with chromatin-remodeling or histone-modifying complexes, such as polycomb repressive complexes (PRCs) and HDACs, they can serve as scaffolds for molecular transport. For example, one such scaffold can mediate the recruitment of PRCs to necessary genomic regions to guide the regulation of transcription (Gangisetty, 2018). During aging, the abnormal expression of ncRNAs results in widespread defects in several chromatin-related processes; these defects imply that

ncRNAs are functionally associated with the stability and integrity of chromatin and, thus, can be implicated in aging mechanisms. Various examples of lncRNA dysregulation have been implicated in AD. For example, BACE1-AS is an abundantly expressed lncRNA within several brain regions of AD patients, which regulates the expression of BACE1, which is critical in AD pathophysiology (Gangisetty, 2018). Other lncRNAs are implicated in the prefrontal association areas and hippocampal regions of AD brains, suggesting a role for these epigenetic modulators in the regulation of synaptic plasticity and strengthening of neural networks.

Recent evidence suggests that epigenetic mechanisms play a pivotal role in neurodegenerative processes, including DNA methylation, histone modifications, and non-coding RNAs. This research has contributed to growing evidence for epigenetic modulation and mediation of risk for AD. An epigenomic review of AD reveals a significant, selective reduction in the expression of genes associated with synaptic plasticity. The loci for epigenetic marks, including chromatin alterations, associated with these expression changes correlate with impaired plasticity and cognitive networks. Furthermore, inflammatory and immune response genes demonstrated increased activation (Gangisetty, 2018). The natural products of epigenetic modifications present a promising treatment strategy for complex, multifactorial neurological disease. Clinical epigenetics seeks to provide insights into the molecular basis of polygenic disorders by narrowing "the biological gap between genetic variation and its functional impact," through such epigenetic moderators (Freytag et al., 2018).

Table 1. Regional distribution of loci which are epigenetically modified in AD pathology (Absalon et al., 2013; Gangisetty, 2018; Li et al., 2008; Lord & Cruchaga, 2014).

Gene	Locus
MIR34A	Chromosome 1 (9151668-9151777)
MIR137	Chromosome 1 (98046070-98046171)
MIR181B	Chromosome 1 (198859044-198859153)
MIR291	Chromosome 1 (155335261-155563160)
S100A2	Chromosome 1 (153561108 -153565830)
TMEM59	Chromosome 1 (54026681-54053573)
MIR128	Chromosome 2 (135665397-135665478)
MIR16	Chromosome 3 (160404745..160404825)
NEP	Chromosome 3 (155024124..155183729)
MEF2C	Chromosome 5 (88699654 - 88922692)
MIR146	Chromosome 5 (160485352-160485450)
MIR106B	Chromosome 7 (100093993-100094074)
ANK1	Chromosome 8 (41653225-41896762)
CLU	Chromosome 8 (27596917-27615031)
ANRIL	Chromosome 9 (21994791-22121097)
MIR24	Chromosome 9 (95086021-95086088)
CDH23	Chromosome 10 (71396934-71815947)
miR-103	Chromosome 10 (89592747-89592827)
Sirt1	Chromosome 10 (67884669-67918390)
miR-107	Chromosome 10 (89592747-89592827)
BACE1	Chromosome 11 (117285686-117316256)
Kcnq1ot1	Chromosome 11 (2608328-2699998)
miR-130a	Chromosome 11 (57641198-57641286)
RB1	Chromosome 13 (48303747-48481890)
miR-496	Chromosome 14 (101060573-101060674)
ADAM10	Chromosome 15 (58595204-58749978)
miR-1538	Chromosome 16 (69565808-69565868)
RPL13	Chromosome 16 (89560657-89566829)
miR-101	Chromosome 17 (72121020-72126420)
RHBDF2	Chromosome 17 (76470893-76501440)
APOE	Chromosome 19 (44905749-44909395)
MIR125	Chromosome 19 (51693254-51693339)
miR-644	Chromosome 20 (34466325-34466418)
miR-645	Chromosome 20 (50585786-50585879)
miR-155	Chromosome 21 (25573980-25574044)
APP	Chromosome 21 (25880550-26171128)
miR-221	Chromosome X (45746157-45746266)

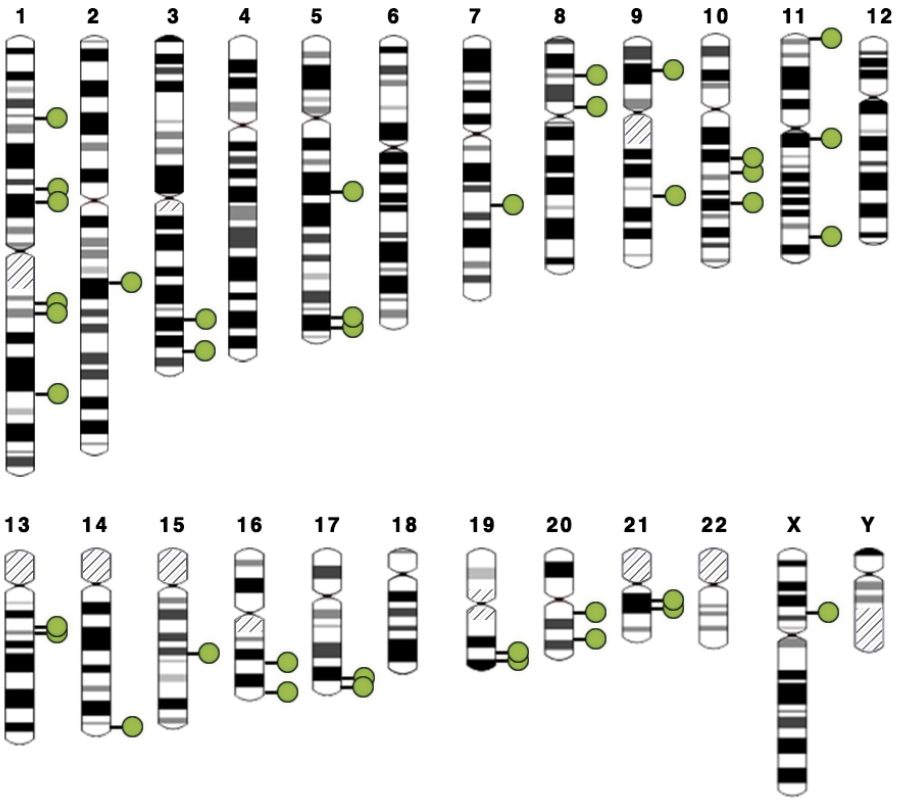

Figure 6. A chromosomal ideogram displaying the most significant epigenetically moderated loci for AD, represented by green dots. Significant loci were determined using the genetic risk score algorithm designed by Chauhan et al. (2015). (Absalon et al., 2013; Gangisetty, 2018; Li et al., 2008; Lord & Cruchaga, 2014) .

The epigenetic interface between environmental and genetic risk factors has developed significantly in the last several years, with growing promise of disentangling complex etiologies. Likewise, the advancement of clinical epigenetic research offers a promising supplement to traditionally-restrictive GWAS studies. The field of epigenetic epidemiology is a new endeavor, with large potential and matched limitations. The intricate composition of neurodegenerative disorders necessitates these alternative research methods, and, although epigenetics has greatly aided the pursuit of diagnostic and therapeutic targets, it fails to holistically encapsulate the diseases on its own. The relative adolescence of epigenomic studies alongside the continual difficulty in recognizing and diagnosing AD makes this research vulnerable but nonetheless important. In this field, confounding age variables and the pathophysiology of preclinical AD are added to an already complex matrix of challenges to this research, joined by measurement variability and small subject

populations. Designing a powerful epigenomic study in humans requires the careful consideration of tissue- and cell-type targets and their respective epigenetic marks.

Despite their limitations, the body of literature and emerging epigenetic studies document a number of genomic regions where changes in epigenetic marks are reproducibly happening in the cortex of older individuals who have accumulated AD-related pathology. Figure 6 displays a genome-wide array of loci that are epigenetically altered in AD samples, compared to controls. Table 1 describes these loci geographically by name. Recognizing these regions as potential loci for disease risk manipulation not only improve diagnostic efforts for complex disorders, like AD, but it also introduces the possibility for longitudinal study that has been a challenge for neurodegenerative disorder. As an age-related disease in very inaccessible tissue, AD research and clinical implications would benefit greatly from being able to detect epigenetic changes in an individual over time. Furthermore, such cues would better inform what is currently known about environmental and lifestyle factors which modulate disease acquisition. Even with a finite number of epigenetic loci, however, such research is not entirely cost- or labor-effective at present. As these technologies develop, accessibility, too, advances; however, at present, the field would benefit from pinpointing a few selected loci for further research and detection, rather than casting such a wide net.

Section V. Neurogenesis and AD Pathology

"Once development was ended, the fonts of growth and regeneration of axons and dendrites dried up irrevocably. In adult centres, the nerve paths are something fixed and immutable: everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree." (Cajal, 1913).

Spanning into the late 20th century, neuroscience held, as one of its central tenets, the "no new neurons" doctrine. It was assumed that all neurons are generated exclusively during prenatal development and very early into postnatal life. In the adult brain, neurogenesis was considered to be nonexistent. The doctrine was initially outlined by Santiago Ramon y Cajal, but its reconsideration did not come until the 1960s and 1970s, when it was experimentally challenged and thrown out, with Dr. Joseph Altman's seminal discovery of thymidine-H3-labelled neurons and neuroblasts in adult rat brains (Altman & Das, 1965; Rodriguez & Verkhratsky, 2011). The discovery of the human brain's ability to produce new neurons came no more than several decades ago, with the observation that the olfactory bulb is able to incorporate newborn neurons throughout adult life. Since then, researchers have learned that particular regions can produce completely immature neurons which are subject to a unique selection process and migration (Lledo et al., 2006). This process, known as neurogenesis, is a useful way to build and repair circuits and construct networks of sharing information, including the passage of short-term memories to long-term storage.

Today, neuroscientists regard neurogenesis to operate primarily in two main areas of the adult mammalian central nervous system: in the anterior region of the subventricular zone (SVZ) along the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus (Rodriguez & Verkhratsky, 2011). These areas are known as neurogenic niches, containing multipotent neural stem cells (NSCs). The NSCs that demonstrate a slow self-renewal go on to produce neural progenitor cells (NPCs) with a

faster-dividing cell cycle. These neural precursors ultimately differentiate into neuroglia or neurons, which can migrate into the cell layers these regions and integrate into local circuitry (Seri et al., 2001). Based on observations in rat models, it is estimated that the healthy processes of adult mammalian neurogenesis contribute thousands of new neurons each day to the hippocampal regions. Growing research promotes the role of new neurons in strengthening pre-existing cognitive networks and promoting olfaction- and hippocampal-dependent learning and memory behaviors. Trouche et al. (2009) demonstrated that newly-integrated neurons within the granule layer of the dentate gyrus are recruited in context-dependent ways, contributing to strengthening memory circuits related to a given stimulus. In studies involving mouse models, environmental enrichment has shown to be very influential in correlating neurogenesis and spatial memory tasks (Gage, 2000). These findings suggest that deficits in neurogenesis may negatively impact the plasticity of the hippocampus and its associated neural circuitry.

Neurogenesis has become a topic of intense study in recent years as neuroscientists interrogate the birth of new neurons in their role within neurodegenerative disease etiology and progression. An exploration of AD pathology would be remiss to exclude a comprehensive look at the hippocampus. Linked frequently to memory encoding, the hippocampus is also associated with affective behaviors and emotions (Jahn, 2013). Lesion-based and knock-out studies into the olfactory bulb and hippocampus show that damage to these areas correlates with common early symptoms for AD, including olfactory deficits and difficulty in declarative memory tasks (Jahn, 2013). It is altogether very possible that the observed neurogenesis alterations contribute to the disease's progressive loss of memory and that ongoing cognitive dysfunction be enhanced by a compromised neurogenic system.

Furthermore, neuropathological staging of protein deposits, including intracellular tau tangles and insoluble extracellular plaques of amyloid-β peptides, show that early sites of aggregate formation include the olfactory bulb and hippocampal formation (De la Rosa-Prieta et al., 2016). These neuroimaging results further highlight these regions as pivotal in neurodegeneration and implicate neurogenesis as a mechanism by which AD pathology develops and progresses.

There exists an unmistakable overlap between the neurogenic dysfunction and the corresponding functional manifestation in disorders like AD. However, AD and adult neurogenesis are not only linked by shared locality of sites where early pathological impairments occur; rather, the two share a number of common molecules, as well, which are utilized in both processes. Newer evidence suggests that several of the molecular players in AD play a role in adult neurogenesis, including the amyloid precursor protein (APP) and presenilin 1 (PSEN1) and their metabolites (De la Rosa-Prieta et al., 2016). In the adult SGZ, expression of PSEN1 and other presenilin variants linked to AD correspond to impairments in microglia proliferation and cell differentiation (Choi et al., 2008). The inactivation of PSEN1 in the forebrains of AD mouse models affected environmental enrichment-induced hippocampal neurogenesis (Feng et al., 2001; Ming & Song, 2011). These animal models and other PSEN1 mutants have exhibited deficiencies in neuronal regeneration, cell differentiation among neural precursors, and impairments of dendritic growth of newborn neurons in the adult SGZ (Li et al., 2008; Ming & Song, 2011). Tau hyperphosphorylation in SVZ striatal neurons and in DG neurons can impair the maturation and network connectivity of newly-formed cells (Hamilton et al., 2010; Rodriguez & Verkhratsky, 2011). These findings suggest that not only are neurodegenerative diseases, namely AD, disorders of tissue

death and synapse decay, but the pathology also incorporates dysfunction in new cell development, differentiation, and migration in cognitive networks.

Because AD, like all other forms of dementia, is a process exclusive to humans, substantial efforts have been directed into designing relevant animal models to reflect the neuropathological, biological, and behavioral alterations seen in humans. The unique properties of neurogenesis and neurodegeneration in humans means there is no perfect animal model for Late-Onset AD. Most of the transgenic experimental mice most closely resemble rare familial variants of AD (Jaworski et al., 2010). Many studies performed on transgenic animals expressing the mutant form of the amyloid precursor protein (APP) result in dysregulated neural progenitor cell proliferation (De la Rosa-Prieta et al., 2016; Rodriguez & Verkhratsky, 2011). These complex changes to neurogenesis associated with AD require many follow-up experiments to clarify, especially since these models represent a limited profile of AD's structure. However, just as clinical researchers put emphasis on the rare forms of AD, studying rare variants in animal models is similarly effective in understanding the underlying mechanisms to AD. Other difficulties associated with modeling neurodegeneration in animals include distinguishing disordered processes from normal aging systems and quantifying neurogenic rates in mice across variable genetic compositions, experimental conditions, and biological markers (Jaworski et al., 2010). Regardless, these models will continue to play an important role in the biological and mechanistic understanding of AD in the coming years. The evidence presented here suggests that the development of animal models underlying epigenetic mechanisms may be most successful in improving our understanding of sporadic AD.

Recent findings suggest that proliferating cells in the dentate gyrus of AD brains do not become mature neurons, nor do they migrate into synaptic networks (Li et al., 2008). Some believe that this represents a protective mechanism, the brain's attempt to maintain healthy networks by not integrating potentially immature or damaged neurons, while others suspect this may be an effect induced by medications older patients receive before death (Li et al., 2008). Perry et al. (2012) more recently confirmed these implications, adding that neurogenic abnormalities in AD would differ across the stages of disease progression. Despite these findings, however, in AD brains, elevated expression of neurogenic marker proteins, including DCX, PSA-NCAM, and NeuroD, were found in the hippocampal regions of the SGZ; this suggests higher levels of neurogenesis occurring in disease cases. To reconcile the findings of increased presence of neurogenic markers yet decreased levels of proliferation in the same regions, Li et al. (2008) suggest that while there is an increase in cell birth, these cells fail to reach maturation and integration into pre-existing circuitry. Because neurogenesis within the dentate gyrus plays a pivotal role in the maintenance of cognitive networks and synaptic plasticity, the functional consequences for impaired neurogenesis these regions would include deficits in learning and memory, common hallmarks of AD.

The observed alterations in neurogenesis for patients experiencing symptoms of epilepsy, stroke, and AD suggest that neurogenesis responds to these conditions (Fitzsimmons et al., 2014); it cannot be ruled out, however, that neurogenesis and its dysfunction may also contribute to the persistence and progression of these diseases. As previously stated, the process of adult neurogenesis is extensively regulated. Some of these regulators include environmental and hormonal factors, such as pharmacological agents,

growth factors, exercise, and stress (Fitzsimmons et al., 2014). Observations by Covic et al. (2010) propose that epigenetic mechanisms exist as sensors of environmental changes and induce small alterations of adult hippocampal neurogenesis. In animal models, spatial exploration and exercise have been linked to methylation activity in the dentate gyrus; environmental enrichment is a well-known stimulus of hippocampal neurogenesis and reinforces the philosophy that exercise and mental activity reduce neuropathological risk.

The overlap in neurogenesis-impacting events and those which predispose an individual for AD draws a link between external factors and neural dysregulation, the crux of epigenetic research. Whether dysfunctional neurogenesis is a product of AD progression or, rather, a contributor to other proteinopathies has yet to be completely resolved. It is also possible that these options are not mutually exclusive, that the epigenetic mechanisms governing neurogenesis are impaired with the start of AD pathology. In essence, biomechanical alterations as a result of AD might influence neurogenic processes, and subsequently impacted neurogenesis processes can feed into disease pathology. These damages to neurogenesis result in defective and unsustainable NSCs, which fail to migrate and blend with pre-existing neural networks, leading to an extensive loss of neurons and subsequent deficits in learning and memory behaviors. Furthermore, this cognitive decline is a notable phenotypic hallmark of AD pathology. In the next section, I will explore the epigenetic mechanisms by which neurogenesis is regulated and suggest possible regions by which epigenetic alterations in AD pathology may be contributing to deficits in neurogenesis.

Section VI. Visualizing Neurogenesis in the Epigenome

Neurogenesis is a complex process, composed of carefully orchestrated events under considerable regulation. Epigenetic mechanisms exercise temporal and spatial control of gene expression to construct networks of organization for cell birth, differentiation, and migration. Many of these mechanisms interpret extracellular and environmental cues, which can induce intrinsic neurogenesis processes. Given the prominent neurogenesis niche in the dentate gyrus, the role of stem cells in disease rescue and repair, and the role of the hippocampus in cognition and its dysfunction, it is reasonable to expect great developments in the research of neurogenesis related to AD pathology and the epigenetic mechanisms pertinent to its etiology. Understanding these intersecting biological systems will advance the knowledge not only of the processes behind disease advancement but of the prospect for regeneration and relevant therapeutic and preventative measures in the AD brain.

The diversity of cellular phenotypes can be attributed, in large part, to epigenetic control of gene expression; this mechanistic control has been critically linked to cellular differentiation (Christensen et al., 2009). The large majority of cells in a given organism share identical DNA sequences; however, detailed epigenetic modulators determine cell types, gene expression profiles, and characteristic phenotypes. In both embryonic and adult neurogenesis, the birth of new neurons can be viewed through the lens of a classic stem cell differentiation process. In this perspective, extracellular environmental cues are read by epigenetic mechanisms; the interpretation of these cues allows for biological processes which precisely determine the spatial and temporal expression of regulators in neural stem cells (NSCs) in their proliferation, differentiation, maturation, and migration (Yao & Jin, 2014). During neurogenic processes, epigenetic modulations underlie the accessibility to DNA and

histone proteins in critical genes, shaping the larger transcriptome. These intrinsic players are highly influential in adult neurogenesis in the SVZ and SGZ and are highly conserved from embryonic neurogenesis. Nevertheless, the impact of extracellular elements, the neurogenic niche, and pathology-induced alterations to the environment cannot be ignored (Lledo et al., 2006; Ming & Song, 2011).

Unpredictable alterations to the epigenome and environment can cause normal DNA methylation or histone acetylation processes to go awry; these changes can induce alterations at a transcriptional level, invoking genes involved in basic processes, like neurogenesis. In the past decade, many epigenetic regulatory mechanisms have been associated with the timing and differentiation of neural stem cell lineages. These mechanisms include cell cycle regulators, transcription factors, signal transducing morphogens, growth factors, neurotrophins, and hormones (Lledo et al., 2006; Yao & Jin, 2014). These same mechanisms have been linked to overall dysfunction and the pathogenesis of neurodegenerative disorders, including AD, Parkinson's Disease, schizophrenia, and autism spectrum disorders (Kauwe & Sawamoto, 2009; Taoufik et al., 2018). The host of overlapping epigenetic mechanisms linked both to neurogenic processes and the development and manifestation of AD imply interactions between the two; these epigenetic coincidences join the geographic conjunction of relevant neuroanatomical regions for AD and neurogenesis. Understanding dynamic changes to neurogenesis across the neural epigenomic landscape over time allows for improved identification to the onset of complex diseases, like AD.

There are many questions yet to be answered in the sphere of neurogenesis. In particular, a topic of great experimental interest has been to examine the location specificity of neuron production, which is limited to the dentate gyrus and the olfactory bulb. While

cells divide in many other areas of the brain, only these two regions give rise to functioning new neurons (Gage & Temple, 2013). The specificity of these processes appeals to an evolutionary biologist perspective, which posits that the ability to re-encode, strengthen, and efficiently retrieve memories acts as an adaptation benefit for humans. In a molecular sense, the birth of new neurons can occur selectively in these locations because the environment mechanistically suits and stimulates neurogenesis. As an environmentally-sensitive and epigenetically-moderated process, neurogenesis can be linked to AD through geographic and functional overlap within the hippocampus. As previously mentioned, engagement with stimuli and the macroscopic environment, induces a proliferation of cells in the hippocampus of mouse models, including the adult dentate gyrus; experiences, learning, and acquiring information about the environment have an impact on survival, and, thus, their encoding as memories suits a biological survival advantage to adapt and learn about one's surroundings. The emphasis of neurogenesis in AD is the result not only of shared neuroanatomical locality (i.e. the hippocampus), but neurogenesis illustrates the epigenetic property of gene-environment interactions, which are increasingly pertinent to growing AD research.

In response to demyelination, such as in cases of multiple sclerosis, Jablonska et al. (2014) show changes in cell differentiation patterns. With a change in the microenvironment, induced by the stripping of myelin in the corpus callosum, neuroblast cells began a coordinated effort of forming oligodendrocytes, the producers of myelin in the central nervous system. These results demonstrate how the adult brain utilizes neurogenesis processes to compensate and recover from damage. Recent research by Ming and Song (2011) highlights the importance of the microenvironment within neurogenic niches. They show that, in addition to fate determination and cell differentiation, the environmental

composition and cues contribute to triggering "self-renewal, proliferation, migration, and maturation" processes in these regions. Little is currently known regarding these mechanisms, but the conclusions efforts currently underway imply that these regulations are conserved from embryonic development through adulthood. These self-renewal mechanisms, however, are not seen in AD brains, suggesting a dysfunction in neurogenesis and the potential failure of these processes to sense environmental dysfunction, as it would in another neurodegenerative or neuropsychiatric condition.

During cortical development, neural stem cells generate the layers of cortex in a precise, inside-out order along a controlled timeline (Yoon et al., 2018). The earliest-born neurons form deep layers of the cortex, while younger cells form the upper layers. Histone methylation has been shown to be an important regulatory mechanism over the correct proportions of inner and outer layers of neurons (Yoon et al., 2018). The critical nature of methylation in corticogenesis has been modeled in knockout experiments in mice. As mentioned, microRNAs often act as fine-tuning mechanisms of gene expression, acting by repressing or inducing mRNA in neural cells; however, miRNAs can also act directly with transcription factors to guide the migration of new neurons. The modulation of signaling molecules in cell proliferation and differentiation implies a crucial role for miRNAs during neurogenesis. Though the complexities of the neurogenesis process are still elusive, the differentiation mechanism is regulated by a number of neurotrophic factors, highlighting miRNAs as an important element to the rise of new cells and their diversity.

Because neurogenesis is regulated by a host of epigenetic mechanisms, it stands to reason that restoration of neurogenic properties in AD pathology be conducted through epigenetics, as well. The coming years promise novel therapeutic interventions for combating

the disease, alongside other neurodegenerative disorders. In preclinical studies, a number of epigenetic-based therapies have shown to alleviate cognitive impairments by promoting and sustaining neurogenesis (Li et al., 2008). A recent study in transgenic mice carrying the human APOE ε4 allele showed that, after environmental enrichment, there was a marked apoptosis of neural progenitor cells (Lazarov & Marr, 2010; Levi & Michaelson, 2007). These results imply that part of the mechanism by which the ε4 allele alters an individual's susceptibility to AD involves a compromised neurogenesis process.

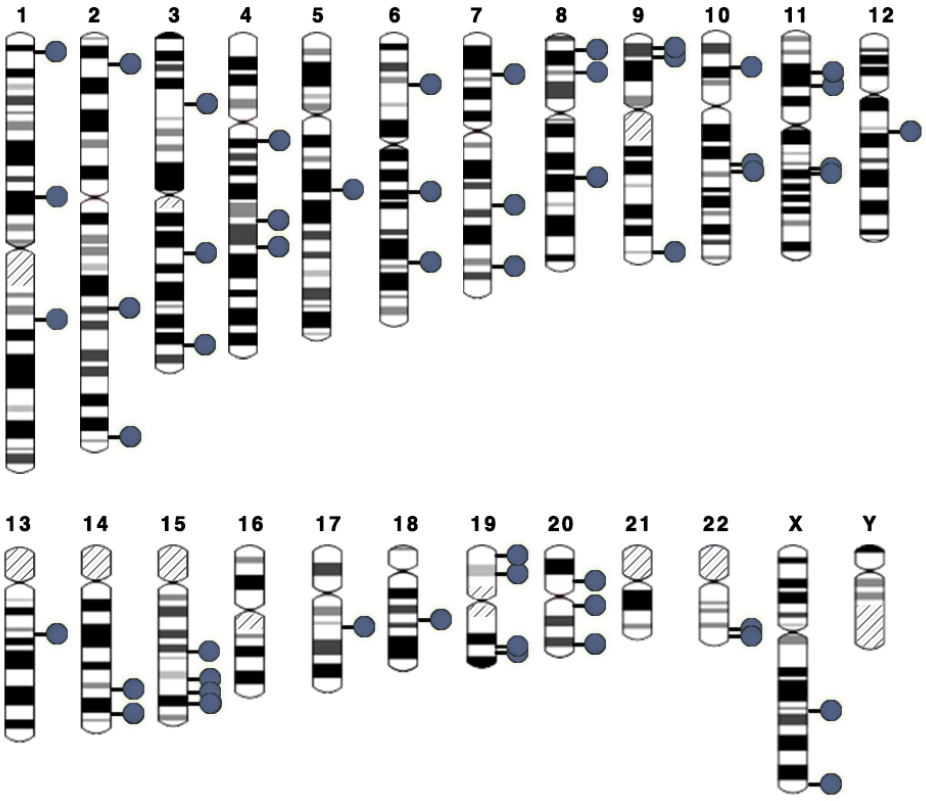

Figure 7. A chromosomal ideogram displaying significant loci implicated in neurogenic processes, represented by purple dots. (Cui et al., 2012; Fitzsimmons et al., 2014; Kaneko & Sawamoto, 2009; Schouten et al., 2012; Yao & Jin, 2014).

Figure 6 and Figure 7 allow for a controlled model of potential epigenetic targets in neurogenesis-affirmed regions of the AD genome. Those epigenetic candidate regions of AD patient research indicate several regions of overlap with genetic regions moderating neurogenesis. This intersection presents a starting block upon which future researcher teams

might launch new epigenetic studies. Using the NCBI's Genome Data Viewer, I have determined and compiled nine unique genetic loci of significance in the AD epigenome which are heavily implicated in neurogenic processes (Fig. 8, Table 2).

Figure 8. A chromosomal ideogram highlighting regions of the genome that are epigenetically altered in AD pathology (green) and loci associated with neurogenic processes (purple); genetic loci which appear in both systems are noted in yellow.

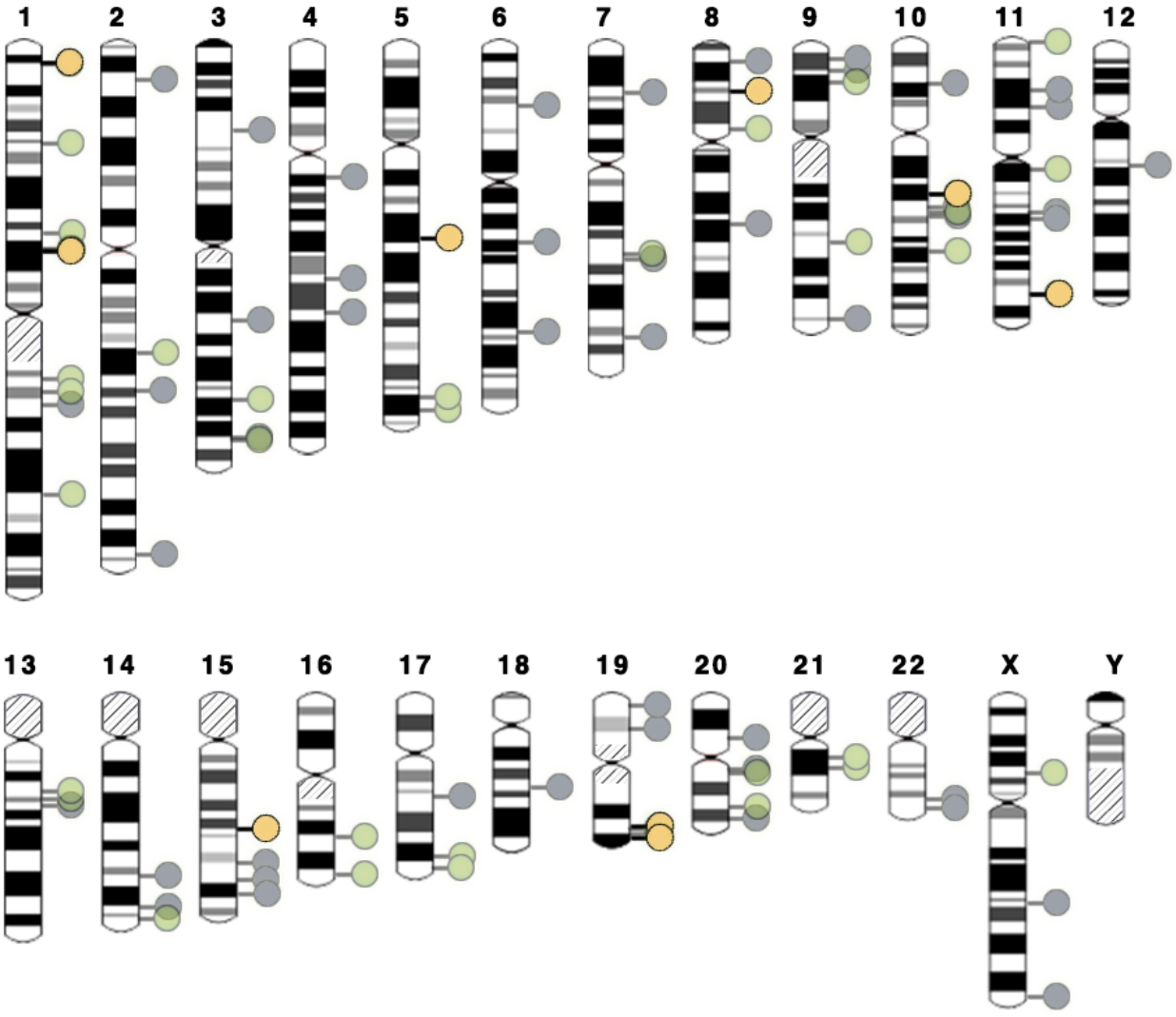

Table 2. Regional distribution of authenticated genetic loci implicated in neurogenesis which are epigenetically altered in AD pathology.

Gene	Locus
MIR34	Chromosome 1 (9151668-9151777)
MIR137	Chromosome 1 (98046070-98046171)
MEF2C	Chromosome 5 (88699654 - 88922692)
CLU	Chromosome 8 (27596917-27615031)
Sirt1	Chromosome 10 (67884669-67918390)
BACE1	Chromosome 11 (117285686-117316256)
ADAM10	Chromosome 15 (58595204-58749978)
APOE	Chromosome 19 (44905749-44909395)
MIR125	Chromosome 19 (51693254-51693339)

Given the inherently interdisciplinary nature of the search for etiological information on neurodegenerative disorders, neurogenesis ought to pose a significant interest to geneticists and neuroscientists alike. Furthermore, because deficits in neurogenesis are hypothesized to occur early in the initial development of AD, a functional understanding might also improve disease outcomes due to earlier diagnosis and therapeutic intervention.

These results fall in line with what is known about the missing heritability of AD after accounting for significant SNPs found in GWAS studies. Ridge et al. (2013) concluded that, outside of major SNPs, chromosomes 1, 4, 5, and 17 are responsible for the most phenotypic variance. These distributions show potential candidates for explaining heritable hallmarks of disease pathology, including several within chromosomes 1 and 5. Because epigenetic mechanisms are altered in AD brains and neurogenesis is regulated by epigenetic modulators, research into the proliferation, differentiation, and maturation of neural stem cells poses a promising target for better understanding the genetic architecture of

neurodegeneration. The continuing advancement of high-throughput sequencing and genome/epigenome editing technologies promises to be a considerable aid in the process of untangling the details of epigenetic regulatory mechanisms, including their impact on neurogenesis. Coinciding developing technologies with longitudinal clinical studies should be of particular interest to those invested in the eradication of neurodegenerative disorders.

Section VII. Conclusion

Analogous to GWAS research, the new generation of EWAS publications has produced a reliable dataset for regions of epigenetic alterations linked to complex traits and disorders. Moving forward, the next steps for epigenomic studies will be to hone in on promising targets for reversing damage and promoting sustainable regulation, several of which have been presented here. In the context of AD, this thesis has presented an attractive new pathological feature moderated by epigenetic markers. It is hoped that this intersection, embodied by neurogenesis, might lead to critical new discoveries and research ventures as epigenomic studies become less expensive and more accessible. These investigations reflect an integral vista point to understand the epigenetic dysfunction mechanisms that hijack the normal aging process into neurodegenerative disorders.

The inflection point in epigenetic research today provides the ability to visualize and contextualize neurogenesis deficits and alterations that occur in AD pathology. Despite the established role of epigenetic mechanisms in neurogenic processes, these neurological and genetic disciplines have largely been applied separately to AD research. Their intersection represents an evidence-based strategy for the two-part quest to better diagnose and recognize complex disorders, as well as apply these findings to a treatment perspective. As such, epigenetics and neurogenesis will continue to serve as areas of interest in the growing research into neurodegenerative disorders. As the body of research grows to accept AD as a disorder which falls under the control of epigenetic mechanisms, the next logical steps in recovering missing heritability will include a focus on potential biological processes that contribute to pathology through such epigenetic pathways. The inclusion of impaired neurogenesis as a hallmark for this disorder is of paramount importance in progressing this

research. Alternative targets, like neurogenesis, contribute to an improving framework for complex age-related disorders. Furthermore, in the case of AD, these targets offer clarification to pre-existing molecular markers for diseases; for example, recent studies have linked neurogenic processes with the development of AD proteinopathies.

Not only is there a substantial body of research recommending the investigation of neurogenesis as an epigenetic moderator for AD risk, but, due to the plasticity of the epigenome, this modulation also serves as an attractive candidate for therapeutic intervention for complex diseases. These interventions can utilize the things which naturally promote neurogenesis, such as physical activity and engaging with the environment. Longitudinal studies suggest that regular cognitive activity and exercise reduce the risk of AD and delay the onset of dementia (Covic et al., 2010; Rodriguez & Verkhratsky, 2011). In a recent study conducted in a transgenic AD mouse model, after a 6-month period of exposure to environmental enrichment, researchers observed both an increased neurogenesis rate and a recovery to normal values of neurogenesis observed in age-matched controls (Rodriguez & Verkhratsky, 2011). The regulation of endogenous neurogenesis promises to be a major target in the development of therapeutic interventions for neurodegenerative disorders, including but not limited to AD. Future work should orient itself in the direction of this formidable intersection and seek to distinguish the effects of epigenetically-modulated systems on AD pathology from normal aging processes, both in animal models and human applications. Furthermore, such experiments must be conducted in order to understand the ways in which environmental factors bear genetic consequences on cognitive networks based on their accumulation of exposure with age.

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APPENDIX A: Glossary of Selected Terms

Acetylation[1] **–** A chemical process by which a hydrogen atom is replaced with an acetyl (CH_3CHO) group, through the use of acetyl co-enzyme A as a group donor

Allele[2] **–** One of a number of variant forms of the same gene in a chromosomal locus. In an organism, each cell contains two copies of an allele for a given genotype. Many alleles are represented with either an upper- or lower-case letter, e.g. "A" or "a"

Chromosome[2] – The highly condensed form of genetic information, containing DNA, histone proteins, and other structural elements, located in a cell's nucleus

CpG site[2] – A location within a DNA sequence in which cytosine and guanine nucleotide bases appear consecutively

CpG island[1] – A region of the genome of one or several kilobases in length, containing a high density of CpG dinucleotides

Declarative Memory[3] – Memory that relates to facts, data, and events, broken into semantic and episodic memory

Epigenetics[2] - The study of heritable changes in genetic material that do not involve changes in the underlying DNA sequence

Epigenomics[6] – The study of genome-wide patterns of changes in chromosomes and chromatin that lead to changes in gene expression

Epigenome-wide association study (EWAS)[1] – A systematic approach to identifying a genome-wide set of epigenetic marks for an underlying trait

Gene[2] – The most basic unit of heredity. Genes represent a segment of RNA or DNA that carries genetic information

Gene expression[2] - The process by which DNA activation and inactivation is converted to functional products, such as proteins production or cell signaling

Genome[2] – The complete genetic content of an organism, often expressed in number of nucleotide basepairs

Genotype[2] – The set of alleles, situated on corresponding chromosomes, that determines a specific trait in an individual. At any one autosomal locus, a genotype will be either homozygous (e.g. "AA" or "aa") or heterozygous (e.g. "Aa")

Genome-wide association study (GWAS)[1] – A method for identifying genetic variants associated with a particular trait which surveys the entire genome for single nucleotide polymorphisms (SNPs) between cases and controls

Haplotype map (HAPMAP)[5] – A haplotype map (HAPMAP) is a catalog of common genetic variants via SNPs. The International HapMap Project seeks to describe patterns of human genetic variation within health and disease

Hippocampus[3] – the cortical structure located in the medial region of the temporal lobe; declarative memories, among many other functions, are encoded by the hippocampus, entorhinal cortex, and perirhinal cortex

Histone[2] - The functional protein that acts as a spool for DNA to wrap around in the process of condensing genetic information into chromosomes. The DNA-histone complex consists of 146 dinucleotide basepairs of dsDNA wrapped around eight histone proteins; this is called a nucleosome

Methylation[2] - The addition of a methyl (CH_3) group

Neural stem cells (NSCs)[4] – These self-renewing, multipotent stem cells can generate both new neurons and glial cells in the nervous system

Neurogenesis[4] – The process of new neuron birth, through NSC activation, proliferation, differentiation and fate specification, migration, and integration into existing circuitry.

Non-coding RNA[2] – The RNA molecules which function to regulate gene expression at the transcriptional and post-transcriptional level. Epigenetically related ncRNAs include miRNAs, siRNAs, piRNAs, and lncRNAs

SNP[2] – A single nucleotide polymorphism or instance of variation between chromosomes by a single base pair

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APPENDIX B: Table of AD Loci Gathered from GWAS

Gene	Chromosome	Function	Risk / Frequency
PSEN 2	1q31-q42	Synaptic plasticity; amyloid-β production; **γ**-secretase activity	Very High / Rare
CR1	1q32	Complement activation; amyloid-β clearance	Low / Common
MTHFR	1p36.22	Neural development; methylation	Low / Common
ECE1	1p36.12	Processing of peptide precursors	Low / Common
CHRNB2	1q21.3	Membrane channel permeability	Low / Common
BIN1	2q14.3	Synaptic vesicle endocytosis; cytoskeletal interactions; APP trafficking	Low / Common
IL1A, IL1B	2q14.1	Inflammatory response	Low / Common
CCR2	3p21.31	Mediates chemotaxis	Low / Common
TF	3q22.1	Mineral transport; filtration and removal of organic material	Low / Common
CXCL8	4q13.3	Inflammatory response	Low / Common
TREM2	6p21.1	Inflammatory response	Moderate / Rare
HLA-DRBS and DRB1	6p21.3	Immune function; histocompatibility	Low / Common
CD2AP	6p12	Cytokinesis; cytoskeletal interactions; receptor-mediated endocytosis	Low / Common
NEDD9	6p24.2	Neural precursor; signal transduction; cell attachment and migration	Low / Common

PGBD1	6p22.1	Unknown	Low / Common
TNF	6p21.33	Cell proliferation, differentiation, apoptosis; lipid metabolism	Low / Common
EPHA1	7q34	Neural development; immune function; synapse development	Low / Common
NME8	7p14.1	Ciliary function; neural cell proliferation	Low / Common
PTK2B	8p21.1	Calcium homeostasis; MAP kinase signaling	Low / Common
CLU	8p21-p12	Chaperone protein; complement regulation; synapse maintenance	Low / Common
IL33	9p24.1	Maturation of Th2 cells	Low / Common
DAPK1	9q21.33	Programmed cell death	Low / Common
TFAM	10q21.1	Mitochondrial DNA replication and repair	Moderate / Common
CH25H	10q23.31	Cholesterol and lipid metabolism	Low / Common
CALHM1	10q24.33	APP processing	Low / Common
CELF1	11p11	mRNA editing; pre-mRNA splicing	Low / Common
MS4A4E	11q12.2	Signal transduction; immune function	Low / Common
PICALM	11q14	Clathrin-mediated endocytosis	Low / Common
SORL1	11q23.2-q24.2	Endocytosis; APOE receptor binding; APP processing	Low / Common
GAB2	11q14.1	Signal transmission	Low / Common

PSEN 1	14q24.3	Intracellular signalling; amyloid-β production; **γ**-secretase activity	Very High / Rare
FERMT2	14q22.1	Cell–cell adhesion; angiogenesis	Low / Common
GWA	14q32.13	Neurite branching; neurite elongation; neuronal migration	Low / Common
MEF2A	5q14.3	Myogenesis; synapse formation	Low / Common
ADAM10	15q22	Hippocampal neurogenesis; cell adhesion	Low / Common
MAPT	17q21.31	Creation of various mRNA species	Low / Common
THRA	17q21.1	Thyroid hormone receptor	Low / Common
GRN	17q21.31	Cell growth	Low / Common
APOE	19q13.2	Synaptic vesicle endocytosis cytoskeletal interactions; lipid transport	High / Uncommon
CD33	19q13.3	Cell signalling; endocytosis	Low / Common
ABCA7	19p13.3	Phagocytosis; lipid homeostasis	Low / Common
LDLR	19p13.2	Protein degradation	Low / Common
BCAM	19q13.32	Cell migration and adhesion	Low / Common
NECTIN2	19q13.32	Inflammatory response	Low / Common
TOMM40	19q13.32	Channel formation	Low / Common
EXOC3L2	19q13.32	Cell membrane dynamics	Low / Common

ENTPD6	20p11.21	Mediation of nucleotidases (NTPases)	Low / Common
CST3	20p11.21	Inhibition of cysteine proteinases	Low / Common
PRNP	20p13	Aggregate mediator	Low / Common
APP	21q21.3	Neuron development; synapse formation and repair; amyloid-β production	Very High / Rare
OTC	Xp11.4	Enzyme encoding of mitochondrial matrix	Low / Common