[Frontiers In Bioscience, Landmark, 23, 1113-1143, January 1, 2018]

Choline, the brain and neurodegeneration: insights from epigenetics

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TABLE OF CONTENTS

1. ABSTRACT

Neurodegenerative disorders are a major public health problem worldwide with huge socioeconomic effect. Recent evidence suggests that neurodegeneration is not only caused by genetic factors but also affected by environmental factors including nutrients. Environmental influences have been shown to cause epigenetic modifications in the brain with long-lasting effects on behavior if they occur in early life. It has been suggested that early nutritional intervention that includes choline, betaine, VitB6, VitB12 and/or folic acid could attenuate decline in cognitive functions. Recently, choline emerged as an essential micronutrient for normal brain development and an epigenetic modifier of the genome that could alter neuronal gene methylation, expression and activity. Choline maintains the structural and functional integrity of membranes and regulates cholinergic neurotransmission via the synthesis of acetylcholine. Choline-related functions have been shown to be dysregulated in several neurodegenerative disorders suggesting a potential role of nutrients in mental health. We will discuss the role of epigenetic mechanisms in neurodegeneration and how nutrients could interact with the epigenome to protect or boost cognitive processes across the lifespan.

2. INTRODUCTION

The prevalence of neurodegenerative disorders is on the rise worldwide. The World health organization (WHO) estimated in 2010 that more than 35.6. million people worldwide have Alzheimer's disease (AD) and projected that this number will increase to 115.4. million by 2050 (WHO). In the US, the Alzheimer's Association (AA) also estimated in 2012 that 5.4 million people have AD and that this number is most likely to increase in 2050 to 11-16 million people if effective treatments during early diagnosis or early prevention are not discovered. This projected increase in the number of people that will develop AD would have adverse effects at many levels, at the individual, social and economic levels. These effects would manifest as an increase in health care cost, a decrease in an individual's productivity and contribution to society and a huge economic burden on society. There is no doubt that these age-related disorders also impose a great burden and stress on afflicted individuals and their family.

The most common neurodegenerative disorders that emerge with aging are Alzheimer's (AD), Parkinson's disease (PD) and Huntington's disease (HD). These disorders are considered progressive, debilitating and multifactorial with a wide

spectrum of symptoms. These symptoms include impairments in cognitive functions, learning and memory decline, attention deficit, sleep deprivation or disturbances, alteration in behavior or swing in mood, loss of productivity, and progressive disconnection from the outside world. These manifestations that are associated with these disorders are often the result of structural, functional and molecular changes in different brain regions of afflicted individuals. Although we progressed significantly in identifying several molecular factors or mediators that are involved in the neuropathology of these disorders, our understanding of such pathologies is limited and our ability to develop effective cure is still far from our reach. This fact necessitates a shift in our focus on how to approach these disorders by implementing effective strategies for early therapeutic interventions and treatment and identifying specific biomarkers to prevent or revert these neuropathologies. These interventions if are introduced at an early stage of the diagnosis of these disorders could impact positively the life of these affected or susceptible individuals and could possibly attenuate the progression of neurodegeneration.

Emerging evidence suggests that not only genetic factors but also environmental factors play a pivotal role in the etiology and/or progression of age-related cognitive and memory decline in neurodegenerative disorders. It has been shown that environmental factors such as repeated exposure to toxins, stress, lifestyle, quality of diet and nutrients that we are exposed to in our life could alter gene expression and function by epigenetic mechanisms resulting in many diseases or neurological disorders (1,2). Many comprehensive studies have shown that nutrition has a great impact on our physical and mental health. Nutrients interact with our epigenome and could negatively or positively impact the expression of many genes in our brain, thus altering brain structure and function throughout life. It is quite remarkable that the influence of these factors during early life could leave long-lasting imprints or marks on the genome, alter when and how much of our genes will be expressed resulting in positive or negative outcomes later in life at all system levels including the brain. Research efforts are now geared toward understanding the contribution of epigenetic mechanisms that are induced by environmental factors such as nutrients in neurodegeneration. Understanding the complexity of interactions between these epigenetic mechanisms, how they work and how they contribute to the etiology of neurodegeneration is quite essential. These efforts could lead to the identification of specific biomarkers for each specific type of neurodegenerative disorder. We may also develop new potential avenues in profiling these epigenetic changes that happen in a degenerating brain then determine how this epigenetic profiling or age-related specific biomarkers would be altered in response to nutrients and the physiological implications.

Advances in the field of epigenetics are revealing complex interactions between our genes and our environment and how these interactions impact our health. For example, nutrition is considered an important environmental factor that has been shown to influence mental health. Studies have shown that supplementation or depletion of specific nutrients during critical period of brain development such as early life and possibly during adulthood when the brain is mature could impact brain structure and function later on life and affect positively or negatively its cognitive functions. Choline has been identified by several studies conducted in animal models and in humans as an important nutrient that could program brain development during early life and affect the genome by causing epigenetic changes in a gene-specific manner or at a global scale in key brain regions that are associated with cognition, learning and memory. What is choline and how does it play a role in brain function? Choline is a nutrient that is required in optimal level throughout life to sustain normal functioning of many organs including the brain. It has both structural and functional roles. It plays a role in the synthesis of acetylcholine (Ach), a neurotransmitter that is required for normal cholinergic neurotransmission in many brain regions (3). It is also an essential structural component of cellular membranes and acts as a precursor for the formation of phosphatidylcholine (PC) and sphingomyelin (SM), two main components of membrane phospholipids (4,5). It has been demonstrated that cholinergic neurotransmission and cellular membrane integrity are two physiological processes that are dysregulated in some neurological disorders including Alzheimer's disease (6–8) suggesting that alteration in the levels of choline in the brain could play a critical role in this disorder. In addition to its role in cholinergic neurotransmission and in maintaining membrane structural and functional integrity, choline emerged recently as an epigenetic modulator of the genome by being a critical factor that alters the methylation status of DNA and histone proteins in the brain, two epigenetic processes that would alter brain function (9). Several studies also showed that choline supplementation during sensitive periods of brain development such as prenatal, perinatal and early postnatal periods could have beneficial effects on learning, memory and on behavior during adulthood (10–14). Prenatal or perinatal choline supplementation may mitigate some of the symptoms that are often associated with neurological disorders including Schizophrenia (15,16), Down's syndrome (17), Rett syndrome (18–21) and Alzheimer's disease (22) and have neuroprotective effects against brain insults including seizures (23–25). Select human studies implicated choline deficiency with the prevalence of decline of mental capabilities with aging

(26–29). This suggests that this nutrient is essential not only for normal functioning of the developing brain but also for the proper functioning of the aging brain and may have beneficial long-term effects on mental health if nutritional supplementation was done early in life and possibly later in life.

Modulation of gene expression and function by epigenetic mechanisms has been linked to many neurological disorders including stress, drug addiction, neuropsychiatric disorders (30–33) and neurodegenerative disorders including Alzheimer's disease (34–36) . This raises the question whether choline and other nutrients that act as "epigenetic modifiers" could be used effectively to attenuate some of the symptoms that are associated with these devastating disorders of the mind. This review focuses on understanding the role of epigenetic mechanisms in the etiology of neurodegenerative disorders and age-related decline in cognitive functions. First, we will discuss the regulation of gene expression and function by epigenetic mechanisms especially in relation to neurological functions. Second, we provide an overview of the physiological functions of choline and its role as an epigenetic modifier of the genome with an emphasis on its effects on brain function. Third, we will discuss how membrane integrity and cholinergic neurotransmission are dysregulated in neurodegeneration. Finally, we will cover research findings that have suggested the potential use of nutrients including choline and the components of the one-carbon metabolism as possible measures to mitigate the adverse effects of aging on memory, learning and cognitive functions.

3. EPIGENETIC MECHANISMS AND NEURODEGENERATION

Several neurodegenerative disease-causing genes have been shown to be altered in expression and function in several areas of the brain by epigenetic mechanisms. Although epigenetic mechanisms are now considered possible instigators for the etiology of these disorders and are emerging as promising mechanisms that are involved, we still cannot establish a causal relationship but a correlative relationship between epigenetic dysregulation of neuronal gene expression and neurodegeneration. To better understand this relationship and how to use this information for treatment, it is important to understand how the epigenetic machinery interacts with the genome and the environment to alter brain function and whether this alteration is specific to each disorder. The epigenetic signature of each neurodegenerative disorder would then be used in order to reveal how this signature could be changed in response to environmental factors and whether this change would have positive or negative outcomes on brain functioning. Consequently, we could then focus on understanding the interaction between genes and those environmental factors that ameliorate or boost brain function.

Cells in the human body have the same genetic code or DNA but they differentiate spatially and temporally into different type of cells with specialized function. This cellular differentiation is governed by a complex network of molecular factors such as transcription factors and downstream signaling molecules to affect the translation of specific genes. It has been shown recently that the expression and activity of several genes in our genome are regulated by epigenetic mechanisms such as DNA methylation, histone protein modifications and non-coding RNAs such as microRNAs. Epigenetic mechanisms play key role in normal development and have the ability to hide or expose regulatory sequences that encode for specific genes along the DNA and in this way modulate the accessibility of transcription factors to these sequences to alter gene expression resulting in long-lasting changes in gene function. The alteration of gene expression by epigenetic mechanisms doesn't lead to changes in the sequence of DNA itself. What is epigenetics? How epigenetic mechanisms work in regulation of gene expression?

Historically, the term "epigenetics" was first defined in 1942 by the British developmental biologist Conrad Waddington as the influence of gene-environment interaction in shaping phenotype and hence behavior. Epigenetics is now more precisely described as a type of molecular and cellular "memory" that results in heritable stable changes in gene expression which are unrelated to changes in DNA sequence and are responsive to environmental factors (37,38). This means that our genetic code is not static but rather dynamic and could be responsive to environmental influences such as stress, physical activity, diet, lifestyle and exposure to toxins. These influences could interact with our genome and epigenome and result in short-term or long-term changes in phenotypes that in some cases could lead to serious diseases (39). It has been shown that these epigenetic changes could be transgenerational if they happen to occur during critical period of brain development but could be reversible. This suggests that specific epigenetic factors that are linked to specific diseases or disorders could have clinical significance and act as potential targets to advance the development of effective therapies for the treatment of specific diseases or disorders (40).

Over the past decade there has been a lot of studies investigating the role of epigenetic mechanisms in age-related neurodegenerative disorders. In the context of the nervous system, epigenetic mechanisms play a critical role in normal brain development and function (41). Epigenetic

dysregulation of gene expression are now regarded as pivotal mechanisms in the etiology of age-related neurodegenerative disorders and a bridge that mediate interactions between genes and environmental factors including diet, lifestyle and physical activity. These mechanisms could cause stable or dynamic changes in neuronal gene expression and function resulting in noticeable changes in phenotypes and behavior that could be long-lasting suggesting the urgent need for the emergence of epigenetics-based therapeutic approaches for the treatment of these disorders. These epigenetic mechanisms are quite complex, are interconnected and are often influenced by environmental factors including nutrients (42). These environmental factors could have beneficial or detrimental effects on all systems in the human body including the brain.

In this section, we will review the role of these epigenetic mechanisms such as DNA methylation, histone modifications and non-coding RNAs especially in relation to the plasticity of the brain and to neurological diseases or disorders. We will focus on changes in these epigenetic mechanisms in neurodegenerative disorders and the potential use of specific epigenetic factors or modulators as therapeutic targets or biomarkers for the treatment of these disorders. We will then discuss the role of choline as an epigenetic modulator and as an environmental factor in modulating the expression of key genes related to cognitive and memory functions and its potential therapeutic applications.

3.1. DNA methylation

DNA methylation is considered one of the best understood types of epigenetic mechanisms. It has long been recognized that DNA methylation is a physiological process that regulates gene expression and function in health and diseased states (43). It plays an essential role in brain plasticity, neuronal survival, and learning and memory functions throughout the lifespan. Gene-specific changes in methylation or global changes in DNA methylation in the brain have been demonstrated in many neurological disorders including neurodegenerative disorders (44) suggesting that methylation as an epigenetic mark should be further investigated in these disorders.

DNA methylation is an epigenetic mark that involves a covalent modification that adds a methyl group (CH3) from the methyl donor S-adenosylmethionine (SAM), which is closely related to the components of the one-carbon metabolism pathway. Dietary intake that influences the one – carbon metabolism such as folate, VitB12, VitB6, choline and betaine could affect DNA methylation (45). Methylation usually occurs at the cytosine (C) residue that is located next to a guanine (G) that is in CpG dinucleotides to form 5-methyl-cytosine (5-mC). These CpGs are found abundantly in the gene promoter and form what we refer to as “CpG islands”. When these CpGs are abnormally methylated, they “lock in” the gene in a silent or repressive state by recruiting other repressor proteins that are linked to gene silencing (46) making it very unlikely for this gene to be expressed (Figure 1). CG dinucleotides were also identified in locations along the gene that are distant from the gene promoter. These CG dinucleotides are referred to as “Orphan CG”. They are “CpG poor” but may play a role in regulation of gene expression.

Methylation is a covalent modification that has been found to be faithfully preserved and transmitted during cell division suggesting that methylation-induced changes in the genome could pass from one generation to the next. Methylation is catalyzed by the activity of DNA methyltransferases (Dnmts) enzymes. These enzymes differ in structure, function, expression and molecular interactions and include Dnmt1, Dnmt2, Dnmt3a, Dnmt3b and Dnmt3L (47). Structurally, these Dnmts share a conserved C-terminal catalytic domain that is made of 10 conserved amino acids motifs and is important for their enzymatic activity except for Dnmt3L. They also contain an N-terminal regulatory domain except for Dnmt2. The N-terminal domain is essential for protein-protein interactions such as interactions of Dnmts with those proteins that are involved in modulation of chromatin structure and function (48). Functionally, Dnmts catalyze the transfer of CH3 from the methyl donor, S-adenosylmethionine (SAM), to carbon C5 of cytosine in the 5’-CpG-3’ dinucleotides. Among these Dnmts, Dnmt1 is responsible for maintenance of DNA methylation that is it maintains methylation tags during DNA replication and cellular divisions. Dnmt3a and Dnmt3b are responsible for de novo DNA methylation that occurs during development and in adulthood. Although Dnmt3L (Dnmt3-like) has no enzymatic activity, it detects along the chromatin unmethylated histone H3 lysine 4 (H3K4) and recruits Dnmt3a to promote methylation of that histone mark to alter gene expression (49). Dnmt2 is mostly involved in RNA methylation (50), a process that is outside the scope of this review and will not be further discussed. In the context of the interaction of environmental factors with our epigenome, diet has been shown to influence the expression and the activity of Dnmts. Several studies conducted in animal models showed that a methyl-deficient diet could alter global or gene-specific methylation and alter the expression and activity of Dnmts (51–54).

The epigenetic mark 5-methylcytosine (5-mC) is now considered an important carrier of epigenetic information and is rarely found at non-CG sites (55). The effect of DNA methylation on gene expression depends on where this methylation occurs along the gene. In most cases, DNA methylation of CpGs in

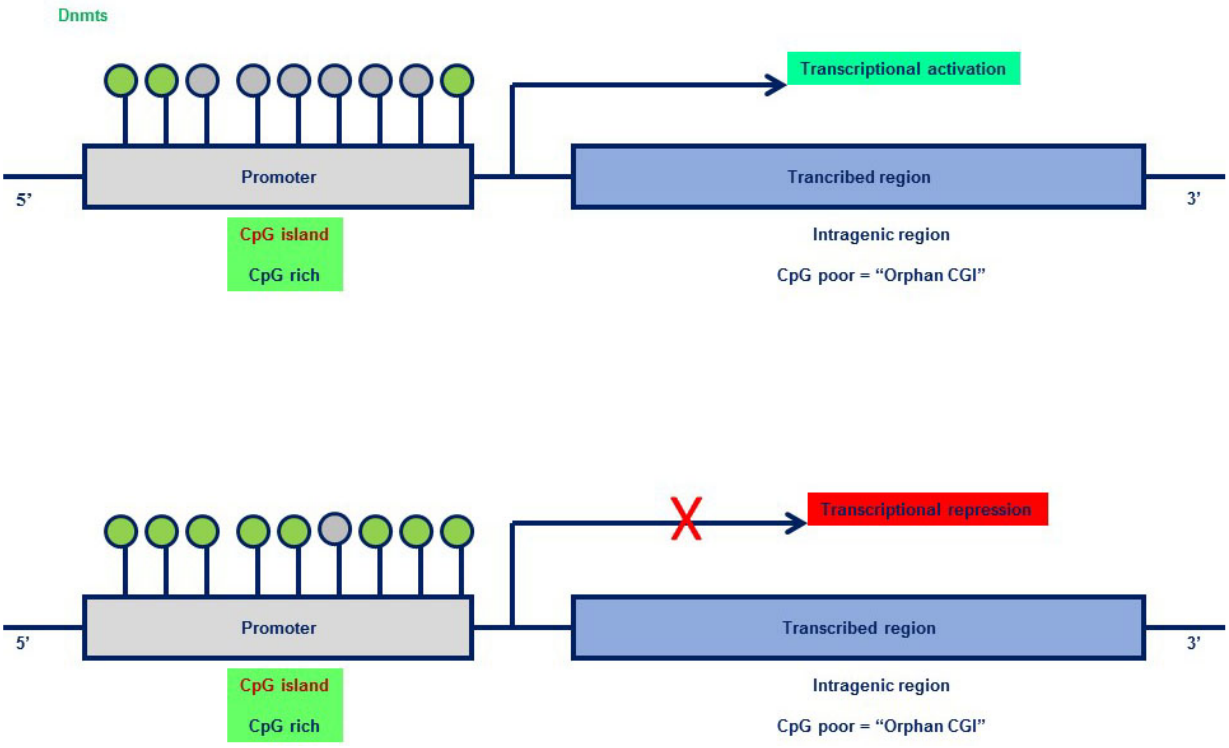

Figure 1. Effects of methylation of gene promoter on gene expression. CpGs are found at a high frequency in the promoter region of the gene. We refer to them as "CpG Island". These CGs are often methylated. Abnormal methylation of these CGs could affect gene expression. An increase in the expression and activity of Dnmts in response to environmental factors could cause a state of hypermethylation of these CpG. This hypermethylation would impede the accessibility of transcription factors to binding site along the promoter and possibly recruit repressors resulting in gene silencing or transcriptional repression. Orphan CGI were also identified along the gene body. These orphan CGs are found in a location that is distant from the promoter region. They are found in intragenic regions. The methylation of these orphan CGI on gene expression correlate with gene activation or repression.

the gene promoter is associated with gene silencing. It does that by prohibiting the binding of essential transcription factors (TFs) to binding sites along the DNA and causing gene repression (56). Methylation of the gene promoter does not only affect the accessibility of transcription factors (TFs) to sequences along the DNA but also recruits repressor proteins such as methyl- CpG-binding proteins such as methyl-CpG-binding protein 2 (MeCP2) and methyl-binding domain proteins (MBDs) to methylated DNA. Methylated CpGs additionally recruit components that alter the chromatin landscape such as histone deacetylases (HDACs), histone methyltransferases (HMTs) and corepressors to create an environment that is conducive for chromatin compaction and gene repression (46,57) (Figure 2). The versatility of Dnmts in being able to interact with the machinery that modulates chromatin architecture make them attractive targets for therapeutic drugs. For example, 5'Azacytidine (5'-Aza), the inhibitor of DNA methylation, emerged recently as a potential agent that could attenuate the adverse effects of some neurological disorders (58). The use of 5-Aza and other Dnmt inhibitors demonstrated that methylation is not a static irreversible process but rather a dynamically regulated process that could be reversible in the developed brain in response to environmental factors. DNA methylation has been linked to many essential neurological functions such as memory formation (59,60), neuronal excitability and synaptic transmission and connectivity (61). Recently, DNA demethylation was detected in mammalian cells and the demethylases such as ten-eleven translocation (TET) family of enzymes (TET1, TET2 & TET3) were identified as factors that convert 5-mC to 5-hydroxymethylcytosine (5-hmC) (62) further proving that methylation is indeed a reversible epigenetic mark that plays a role in neuroplasticity. In the context of neurodegenerative disorders, an elevation in the global levels of 5-hmC with no decrease in 5-mC or change in the expression of TET enzymes has been demonstrated in the dentate gyrus and the CA1 region of the hippocampus of old mice compared to young mice (63). The functional implications of such change in the level of this epigenetic mark and the role of the components of the DNA methylation machinery in the aging process should be further investigated.

It was originally thought that DNA methylation is an example of a stable epigenetic mark that is irreversible. However, several studies showed that this mark is indeed reversible and could be modulated by environmental factors including nutrition with long-

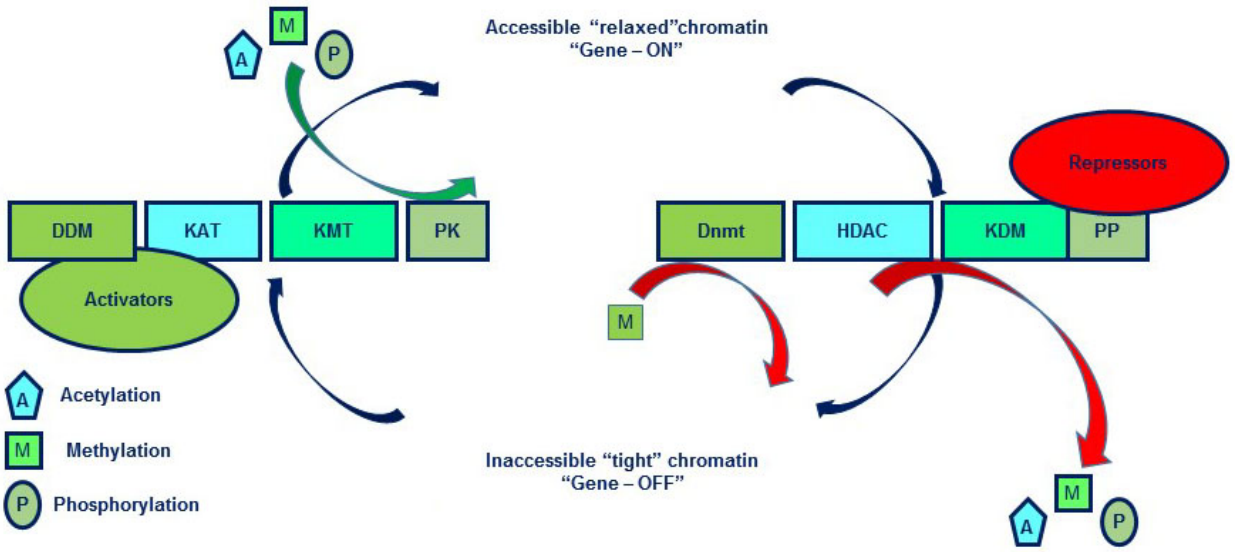

Figure 2. The role of the epigenetic machinery in regulation of gene expression. The regulation of gene expression is quite an intricate and complex process that requires the orchestrated effort and interactions between many players. Malfunctioning or modulation of these interactions could place the transcriptional or translational process out of tune. Acetylation of histones by KAT, phosphorylation of histone H3 and methylation of histone H3 at specific residues are conducive to creating a "relaxed accessible chromatin" and activates gene expression (Gene is ON). Methylation by Dnmts, an increase in deacetylation by HDACs and dephosphorylation of histone H3 by phosphatases provide an environment that is conducive to creating a "tight inaccessible chromatin" and deactivate gene expression (Gene is OFF). KAT=Histone acetyltransferase, KMT=Histone methyltransferase, HDAC=Histone deacetylase, KDM=Histone demethylase, DDM=DNA demethylase, Dnmt=DNA methyltransferase, PK=Protein Kinase, PP=Protein Phosphatase

lasting effects on health. In light of the importance of DNA methylation in many physiological processes, abnormal DNA methylation has been identified in many neurological disorders (64) and neurodegenerative disorders like Alzheimer's (AD), Parkinson (PD) and Huntington (HD) diseases. The recent consensus is that environmental factors including nutrients, diet, lifestyle, physical activity, exposure to toxins, stress, aging and social factors could have equal or even more impact than genetic factors in the etiology of these disorders (65). This raises the question whether early nutritional intervention could protect the brain from insults or could attenuate symptoms that are linked to aging including decline in cognitive functions and dementia.

Epigenetic mechanisms have been linked to many identified AD-related molecular mechanisms. AD is a complex disorder that is most likely resulting from a complex interaction between genetic and environmental factors (66) suggesting that therapeutic approaches that target or modulate the activity of the component of the epigenetic machinery could be promising for treatment of AD. The main brain regions that have been shown to be affected by degeneration in AD disorder are the frontal, temporal and parietal lobes in the cortex, entorhinal cortex, cingulate gyrus and the hippocampus (67). Post-mortem brain tissues of AD patients revealed gene-specific changes in methylation or global changes in methylation in the brain. Several findings revealed an overall decrease in DNA methylation in the AD brains and more specifically a state of hypomethylation of the amyloid precursor protein (APP) gene promoter. This hypomethylation correlated with an upregulation in the expression of the APP and an increase in beta-amyloid deposition in specific areas of the brain (68). Other studies did not find changes in the methylation status of the APP gene promoter. The inability to replicate these findings could perhaps be explained by the differences in sampling and whether different findings were attributed to different types of AD cases. Interestingly, in the neocortex, occipital cortex, parietal cortex, temporal cortex, the hippocampus and in the putamen, a significant reduction in SAM with an elevation in SAH levels were reported in AD cases (69,70). Deficiency of folate and elevation of homocysteine could result in hypomethylation (71) and SAH elevation is known to be a major inhibitor of Dnmt activity which could also lead to a state of hypomethylation in the brain. These findings suggest that nutrients such as folate, VitB12, VitB6 and choline, which participate in the formation of SAM, could be used as supplements to lessen the severity of AD symptoms. A limited study conducted in humans showed that the use of a nutritional supplement that includes folate and the VitB complex could delay the progressive decline in memory and improve overall performance in specific tasks in AD patients (72,73).

PD is another common neurodegenerative disorder that is characterized by the loss of dopaminergic neurons in the midbrain and substantia nigra, brain regions that are mostly involved in regulating motor functions. One of the hallmarks of this disorder is the accumulation of cytoplasmic protein aggregates including alpha-synuclein. This protein is encoded by the alpha-synuclein gene (SNCA). Its accumulation in diverse brain areas leads to progressive neuronal loss with time (74). Although genetic factors have been identified as the causative agent for this disorder, environmental factors have also been suggested to be additional main players in the etiology of this disorder (75). Modulation in the activity or levels of the components of the one-carbon metabolism has also been linked to PD. Animal models of PD showed that deficiency in dietary folate elevated plasma levels of SAH compared to SAM and increased the vulnerability of dopaminergic neurons and increased oxidative stress in a mouse model of PD (76). Changes in methylation was also detected in some PD-related genes. For example, post-mortem brains of PD patients revealed a significant decrease in the methylation of intron 1 of the alpha-synuclein (SNCA) gene compared to controls in several brain regions such as the substantia nigra, putamen and cortex. This decrease in methylation correlated with an increase in alpha-synuclein gene expression, its accumulation and its contribution to the the progression of PD pathology (77). In addition to changes in gene expression, hypomethylation in SNCA gene promoter correlated with a decrease in the expression and activity of Dnmt1 in postmortem PD brains (78). Other studies did not replicate this finding. Additional epigenomic studies of these neurodegenerative disorders are required.

3.2. Histone Posttranslational Modifications

Epigenetic mechanisms also include complex post-translational modifications of the protein histones that package the chromatin. The DNA packages itself around octamer of histones forming in this process nucleosomes. There are 5 different types of histones: H3, H4, H2A, H2B and the linker histone H1. Each octamer consists of 2H3, 2H4, 2H2A and 2H2B whereas the linker H1 joins the nucleosomes. Structurally, each histone consists of a globular domain and a charged NH2-terminus tail that protrudes out of each histone. The switch between chromatin compaction and chromatin relaxation states is regulated by the ability of the histone tail to perform malleable posttranslational modifications (PTMs) at specific amino acid residues along this tail. These PTMs are quite diverse and constitute a “histone code”. These modifications are not random but occur at specific residues along the tail such as on lysine (K), arginine (R), serine (S) or threonine (T) and accordingly can alter the accessibility of TFs by hiding or exposing regulatory sites along the DNA (79,80) allowing the chromatin to be in a condensed form (heterochromatin) or in a decondensed/relaxed form (euchromatin). These dynamic PTMs has been shown to occur in dividing cells as well as in adult post-mitotic neurons (81). A variety of histone modifications has been identified and mostly include methylation, acetylation, phosphorylation, sumolylation or ubiquitination. These modifications often target histones H3 and H4 which have central role in chromatin structure and function (79,82). The most widely studied and understood PTMs are methylation, acetylation and phosphorylation. Histone modifications have been observed in some neurodegenerative disorders. How do these PTMs affect gene expression? What are their physiological implications in neurodegeneration? Could they have therapeutic potentials for the treatment of neurodegeneration? We will describe selected PTMs that have been shown to alter gene expression then we discuss selected studies that showed changes in PTMs in some neurodegenerative disorders.

In particular, lysine methylation and acetylation play critical role in transcriptional regulation (83) (Figure 2). Histone methylation is a biochemically static and inert process that is catalyzed by the enzymatic activity of histone methyltransferases (HMTs/KMTs) that utilize S-Adenosylmethionine (SAM) as a methyl donor and histone demethylases (HDMs) that removes these marks. This histone methylation involves the addition of the uncharged methyl group (CH3) to lysine (K) or arginine (R) residues of H3 and H4 without altering their charge. When methylated, these residues along the histone tail act as “nucleation site” for recruitment of effector proteins along the gene (84). The effects of histone methylation on chromatin structure and on transcriptional regulation of gene expression are quite complex. The outcome of histone methylation on gene expression depends on which amino acid residue of a specific histone is methylated and how many methyl groups are added. It has been demonstrated that histone H3 at lysine 4 (K4) or histone H3 at lysine 9 (K9) can be mono (me1), di-(me2) or tri-(me3) methylated on their amine (85,86).

Histone acetylation is another histone modification that plays a dynamic role in chromatin remodeling and in gene expression regulation. It is a specific and reversible modification that is regulated by the activity of histone acetyltransferases HATs (KATs) and histone deacetylases (HDACs). Four classes of HDACS (HDACI, II, III, IV) have been identified. Each class contains different subclasses. These HDAC subclasses are differentially expressed in the human body. Acetylation involves the transfer of an acetyl group from acetyl-coenzyme A to the NH2-terminal tail of lysine residue (87,88). HATs/KATs and HDACs work on nucleosomes that are situated next to a TATA box to which RNA polymerase II binds and hence assist

in transcriptional activation or repression (89). Unlike lysine methylation, lysine acetylation is not an inert modification. Via its negatively charged acetyl group, it reduces the positive charge on the N-terminal tail of H3 and reduces the electrostatic interaction of this tail with the negative phosphodiester bond of DNA. This results in loosening histone-DNA interactions and creates an open permissive relaxed chromatin (euchromatin) and an environment around DNA that is conducive for transcriptional activation (87,90).

Histone phosphorylation is also a well-studied PTM. This PTM is mediated by the activity of kinases and phosphatases and it is a key component for chromatin compaction during cellular division. Phosphorylation of H3 occurs at serine (S), threonine (T) or tyrosine residues. These three histone modifications that we already discussed influence each other in a synergistic or antagonistic manner and influence DNA but they cannot solely modulate gene expression and function. They are highly interconnected, interact with the DNA methylation machinery and form an elegant regulatory system to modulate gene expression and function (91).

Studies on changes in histone modifications in the brain with neurodegeneration are scarce but emerging. Selected human studies identified changes in specific histone marks or changes in the levels of the enzymes that are involved in these PTMs in specific regions of AD postmortem brains. For example, a significant decrease in the protein levels of HDAC6, a deacetylase that interact with tau protein and influence its phosphorylation and accumulation, was reported in the cerebral cortex and hippocampal tissues of AD postmortem brains (92). In the context of other histone modifications, an increase in the repressive mark trimethylation of histone H3 at lysine 9 (H3K9) was reported in the temporal cortex and hippocampus of AD postmortem brains. Phosphorylated histone H3 was also detected in the cytoplasm of hippocampal neurons of AD brains and this correlated with the presence of phosphorylated tau in these neurons suggesting that this epigenetic modification which is usually restricted to the nucleus was found in the cytoplasm and may be a cause of neurodegeneration in AD (93). Although all these identified changes in histone marks correlated with the pathology of this neurodegenerative disorder, further studies are needed to understand the physiological implications. Profiling global changes of specific histone marks as well as changes of histone marks in specific types of neurons that are vulnerable to degeneration are required.

HDAC2 is another histone deacetylase that has been shown to reduce the acetylation of learning and memory-related genes. For example, animal studies revealed that an elevation in the levels of HDAC2 has been linked to memory decline that is often associated with neurodegenerative disorders. The use of an HDACi (inhibitors of HDACs activity) could lift this repression on these key genes related to memory and enhance cognitive capabilities in neurodegenerative disorders (94). Other animal studies showed that the use of an HDACi such as sodium butyrate was able to improve learning or attenuate memory loss and cognitive deficits in animal models of AD (95,96). This correlation between the use of HDACi and behavioral changes are not very clear but suggests that the role of the components of the epigenetic machinery should be further studied in order to determine if they have future therapeutic potential and could act as targets for drugs for treatment.

3.3. Non-coding RNAs

Non-coding RNAs such as microRNAs are now emerging as key players in regulation of gene expression and in neurodegeneration. These intriguing elements are highly conserved small 18-22 nucleotide long non-coding RNAs that do not code for proteins but do regulate the expression of many protein-coding genes (97,98). In the context of brain function, these elements play a role in regulating neuronal gene expression in a spatial and temporal manner (99). They are abundantly expressed in developing and mature brain and modulate gene expression at different stages of neuronal development in diverse organisms (100,101) suggesting their role in neuronal survival and aging. These regulatory elements are used as biomarkers for early diagnosis of diseases and could have the potential in becoming biomarkers for specific types of neurodegenerative disorders. We do now have the technology to profile the changes in the expression of these elements in response to environmental factors. That is why, it would be necessary to profile changes in the expression of miRNAs in the blood or CSF of patients in the early stages of neurodegeneration. Early identification of specific biomarkers could help us in better understanding their roles in neurodegeneration and to develop effective strategies in treatment by promoting interventions at early stages of neurodegeneration.

How these epigenetic mediators work? These regulatory molecules can fine-tune the protein output and its function in a very fast, efficient and reversible manner, even in restricted neuronal compartments including dendrites and dendritic spines (102). Guided by the RNA-induced silencing complex (RISC), they usually regulate gene expression at the posttranscriptional level by recognizing the miRNA recognition element (MRE) in the 3'- untranslated region (3'-UTR) of their target gene. It has been demonstrated that miRNA regulation of gene expression is dependent on the complementary match between the seed region of miRNA (2-7 nucleotides) and the 3'-UTR of the target gene (Figure 3). This

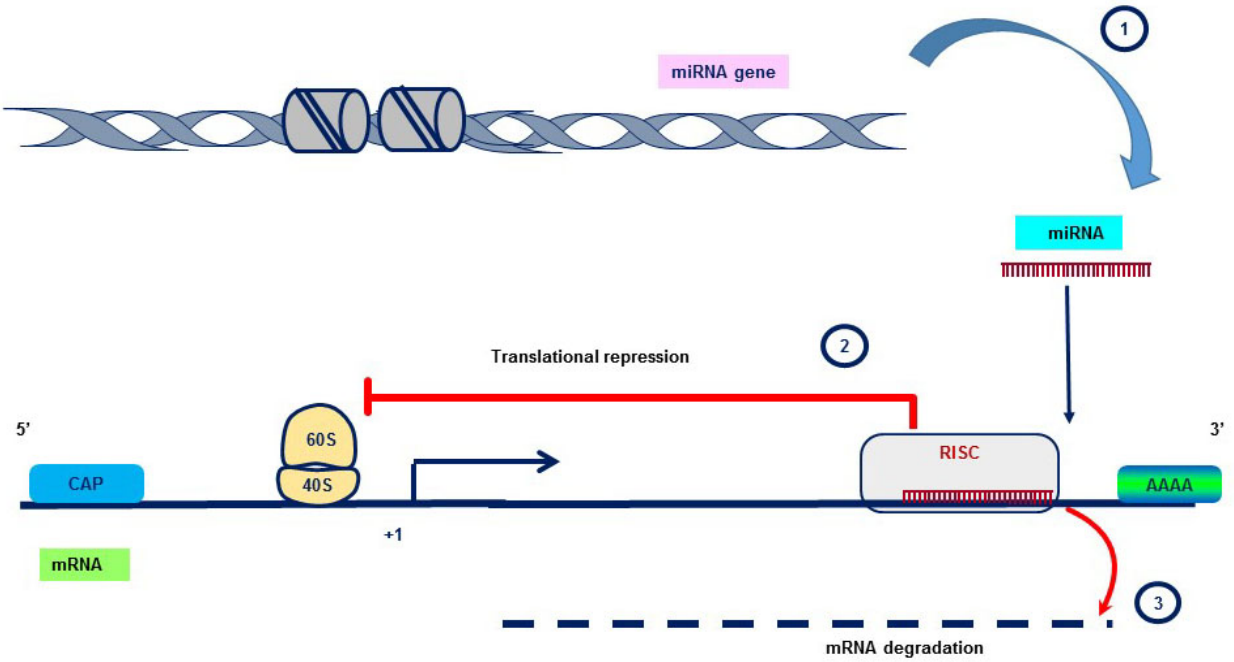

Figure 3. Conceptual diagram of the effects of miRNAs on regulation of gene expression. miRNAs come from a miRNA gene. miRNAs bind by base complementarity to the 3'UTR of a target gene causing translational repression. miRNAs could also bind to the 3'UTR of a gene causing mRNA degradation (Modified from 217).

complementarity most often results in mRNA cleavage and/or in translational repression of the mRNA (103) thereby making these elements potentially very important players in neuroplasticity. Other studies showed that miRNAs could in some cases induce gene expression and mRNA translation (104). Interestingly, these regulatory elements interact with other epigenetic mechanisms and their expression could also be regulated by epigenetic mechanisms such as changes in the methylation of their promoter or by chromatin remodeling. miRNAs have been shown to play a role in altering neuronal communication (105) and have been implicated in many diseases including neurodegenerative disorders (106).

MicroRNA profiling revealed differential expression of these regulatory elements in AD patients and AD animal models compared to controls. This differential expression in miRNAs was detected in the blood and in the cerebrospinal fluid suggesting that miRNAs could be used as biomarkers for specific types of neurodegenerative disorders (107) and that early screening of these biomarkers could potentially be helpful for early interventions. The expression and function of alpha-synuclein gene (SNCA), a PD-related gene, has been shown *in vivo* and *in vitro* studies to be regulated by microRNA-7 (miR7). miR-7 repressed the expression of this protein by targeting the 3'UTR of SNCA mRNA, decreasing its expression resulting in decreased toxicity of this protein in dopaminergic neurons (108). The abundantly expressed miRNA in the brain miR-124, which has been shown to play a role in neuronal differentiation and neurogenesis, was found to be reduced in a mouse model of PD (109) raising the question of the role of miR-124 in the etiology of PD. These selected studies suggest that miR-7 or miR-124 could be used as potential PD biomarkers. Other studies showed that the profiling of miRNAs in the brain of HD individuals and in HD animal models revealed dysregulation in their expression compared to normal brains (110). Future studies should aim at profiling the changes in miRNA expression in neurodegenerating brain and compare it to that of a normal brain. This would most likely identify specific biomarkers that could be used as "early signature" for specific type of neurodegenerative disorders. This information would then promote faster and effective strategies for early treatment to decrease the worsening of these disorders with time.

3.4. Choline as an epigenetic modulator of the genome

Choline can donate its methyl groups by converting into betaine. In doing so, it can participate in folate-mediated one- carbon metabolism and participate in the formation of the major methyl-donor SAM. SAM provides CH3 to the components of the epigenetic pathways such as Dnmts for DNA methylation and HMTs for histone protein methylation

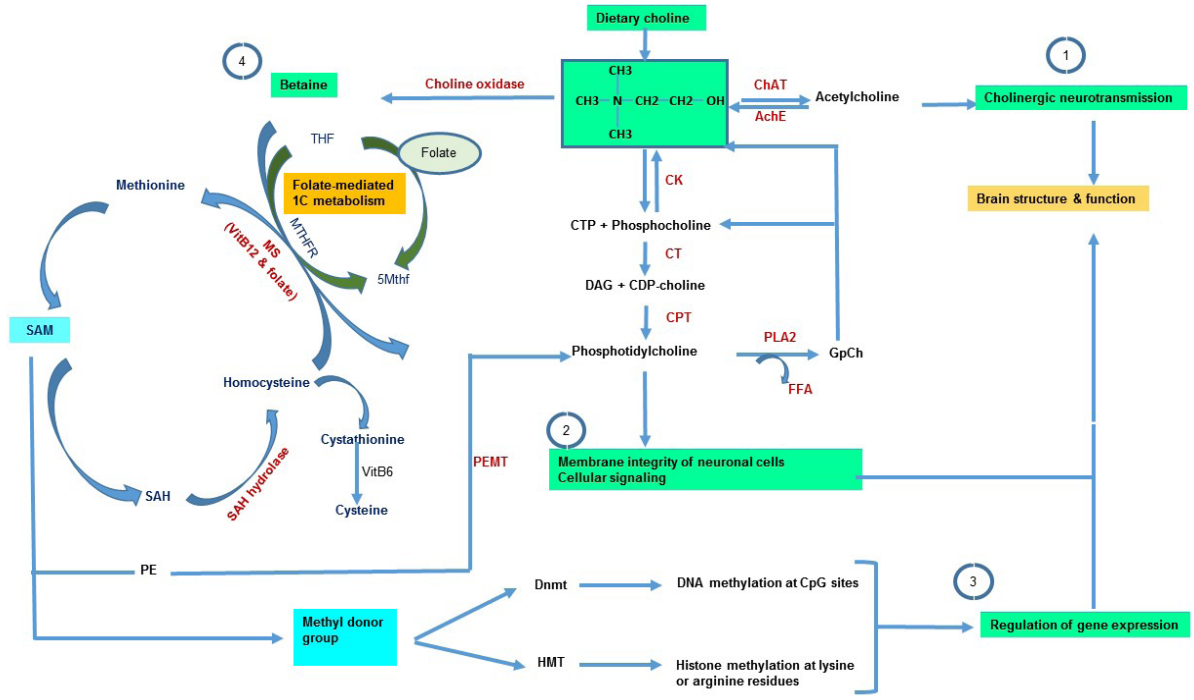

Figure 4. Choline has many essential physiological functions: 1) it plays a role in the synthesis of Ach and in regulating cholinergic neurotransmission in specific brain regions. 2) Choline is a major precursor for the formation of membrane phospholipids such as PC. 3) Via SAM, it acts as a major methyl-donor for Dnmts and HMTs, enzymes that catalyze DNA methylation and histone methylation to regulate gene expression. 4) Via its derivative betaine, it plays a major role in the folate-mediated 1C metabolism. VitB12, folate and VitB6 play important roles in SAM metabolism. After donating its methyl group, SAM is converted to SAH which is identified as a major inhibitor of the Dnmt activity. Via the activity of SAH hydrolase, SAH can be converted to homocysteine. Homocysteine can be remethylated back to methionine via the transfer of a methyl group from 5Mthf by methionine synthase (MS) which requires the cofactors VitB12 and folate for its activity. Homocysteine can also be converted to cystathionine. AchE = acetylcholinesterase, ChAT = choline acetyltransferase, CK = choline kinase, CT = CTP:phosphocholine cytidyltransferase, CPT = CDP-choline:1,2,diacylglycerol cholinephosphotransferase, PLA2 = Phospholipase A2, FFA = free fatty acid, PEMT = Phosphotidylethanolamine N-methyltransferase, MS = methionine synthase, 5Mthf = 5-methyltetrahydrofolate, MTHFR = Methyltetrahydrofolate reductase, THF = Tetrahydrofolate, SAH = S-adenosylhomocysteine, SAHH = S-adenosylhomocysteine hydrolase, DNMT = DNA methyltransferase, HMT = Histone methyltransferase.

(Figure 4). Methylation of genes or histones has been shown to modulate gene expression and function in a spatial and temporal manner in the brain. Variation in choline levels in the developing and the mature brain has been linked to many neurological disorders implicating the role of this nutrient in mental health. It is then not surprising that a choline-deficient diet or a choline-supplemented diet could modulate epigenetic marks on genes and alter their function in specific brain regions. We describe here recent knowledge about the epigenetic role of choline in the brain and describe how alteration in the level of this micronutrient during early development and possibly later in life could be linked to the neuropathology of some neurodegenerative disorders that appear in some individuals with aging.

Diet is a main modulator of the epigenome throughout life with prominent effects during early development. Early life is a critical period that is characterized by rapid cellular growth and differentiation. Exposure to environmental factors during this critical period could program brain development and result in long-lasting changes in brain function. So, a healthy diet is a foundation for a good mental health throughout our life and particularly vital during early life when the brain is undergoing excessive neurogenesis, neuronal differentiation and migration and establishment of neuronal networks. Diet that is rich in components that participate in the one-carbon metabolism and the folate cycle is essential for normal development of the brain (Figure 4). These components include choline, betaine, methionine, folic acid, VitB12, and VitB6 and participate in methylation reactions to regulate neuronal gene expression by epigenetic mechanisms. Animal studies showed that supplementation of choline during prenatal life increased neurogenesis and angiogenesis in the fetal hippocampal region and improved animals' performance in memory-related tasks that lasted for a lifetime (14,111). These studies suggest that providing favorable environmental factors including an optimal diet during critical period of brain development could boost the activity of the brain later in life. Other studies showed that dietary choline deficiency decreased the levels of SAM in the brain resulting in a state of hypomethylation. For example, *in vitro* studies revealed that prenatal choline supplementation changed fetal expression of

the cell cycle inhibitor CDKN3 in the dentate gyrus in the hippocampus by epigenetic mechanisms. On the other hand, choline-deficient mice had a hypomethylated CDKN3 gene that resulted in an increase in the expression of this gene and an increase in its activity by inhibiting the cell cycle progression and hence neurogenesis. Choline-induced changes in CDKN3 gene methylation status and function suggest that choline modulates the expression of cell cycle regulators and hence affects neurogenesis, cell proliferation and brain development and function (53). These findings conducted in animal models were also confirmed in select human studies. It has been demonstrated that prenatal choline supplementation prevents neural tube defects (112). Choline-related compounds such as CDP-choline have been shown to enhance some form of memory and cognitive functions with aging (113,114).

Prenatal choline supplementation also resulted in an increase in the levels of trophic factors later in life. An elevation in the levels of brain-derived growth factor (BDNF), neurotrophic growth factor (NGF) such as neurotrophin-3 (NT-3), and Insulin growth factor (IGF) and vascular endothelial growth factor (VEGF) was reported in the hippocampus of choline-supplemented rats with aging (115,116). It also resulted in anatomical changes in brain areas that are critical for learning and memory. For example, choline-supplemented rats showed an increase in the size of cholinergic neurons in the basal forebrain and in their ability to release Ach at synapses (117). These studies indicate that the availability of the nutrient choline during early life such as prenatal or perinatal could program brain development and induce long-lasting positive effects on cognitive abilities during adulthood and with aging.

In addition to changing DNA methylation, prenatal or perinatal choline supplementation has been shown to alter the methylation status of histones and affect the expression of key genes related to fetal brain growth and development. The prenatal and postnatal neuroprotective effects of choline are discussed in details in other sections of this review. These dynamic processes such as methylation and histone modifications could also happen in the mature brain and alter its function which prompt the idea that nutritional intervention that includes choline and other nutrients may protect cognitive functions with aging. So there is an urgent need to have an epigenomic profiling of specific types of neurodegenerative disorders in order to identify how and what environmental factors including nutrients could influence gene function in specific type of disorder and what can be done earlier in terms of nutritional supplementation to prevent brain insult later in life or delay the worsening of neurodegenerative disorders.

4. CHOLINE PHYSIOLOGICAL FUNCTIONS

Choline (2-hydroxyethyl-trimethyl-ammonium) is an essential nutrient that has three methyl groups attached to the nitrogen atom of ethanolamine (Figure 4). Choline serves as an essential methyl donor in methylation reactions that occur via the betaine-methionine pathway. Choline acts as a precursor of several phospholipids in the mammalian brain such as phosphotidylcholine (PC), phosphotidylethanolamine (PE) and sphingomyelin (SM) (118). These choline-containing phospholipids have essential physiological functions including membrane biogenesis, cellular signaling, nerve cell myelination, cellular division and lipid transport, all are processes that are required for proper development and normal functioning of the brain. Choline is also a precursor for the neurotransmitter Ach. Ach is the main neurotransmitter that is released by cholinergic neurons in various areas of the brain to regulate cholinergic signaling or neurotransmission (119), a function that is often dysregulated in some neurodegenerative diseases such as Alzheimer's disease and is characterized by cognitive impairments (120,121). Ach also plays roles in synapse formation and neurogenesis. Therefore, choline-containing compounds could provide a safe approach for therapy and should be further investigated as other viable options for treatment of neurodegenerative disorders in the early stages of prognosis. What are our sources of choline and how does the brain get its pool of this nutrient?

Although choline is derived from the diet, several people do not get the optimal requirements that are needed for intake which is 7.5. mg daily per kg of body weight. This optimal value has been shown to vary between individuals in response to several factors such as sex, age, genetic polymorphisms and environmental factors (122,123). For example, the adequate intake of choline is often elevated in pregnant women who require additional amount of this nutrient to support fetal growth and development. Compared to men, non-pregnant women require lesser amount of choline because of the ability of estrogen to perform de novo synthesis of this essential nutrient. Our sources of dietary choline are often derived from wide variety of foods including eggs, peanuts, liver, wheat germ, beans, fish, some meats and vegetables. After ingestion of choline-rich foods, the plasma levels of choline are elevated. Choline in circulation can cross the blood brain barrier (BBB) and be metabolized further in the brain or could be oxidized mainly in the liver into betaine to serve as a major source of methyl groups in this organ.

In addition to obtaining choline from diet, choline can also be de novo synthesized from PC by a series of complex chemical reactions that have been

shown to occur primarily in the liver and to a lesser extent in other tissues as well including the brain. In mammals, the de novo synthesis of choline is catalyzed by the enzymatic activity of phosphotidylethanolamine-N-methyltransferase (PEMT) via the sequential methylation of phosphotidylethanolamine (PEM) to form phosphotidylcholine (PC) using SAM as a methyl donor (124). Much of the dietary choline that reaches the liver can be converted in an irreversible reaction into betaine via the enzymatic activity of choline oxidase. This oxidation provides a source of methyl groups for the formation of methionine and SAM, major methyl-donors for methylation pathways (Figure 4). A small portion of dietary choline is usually acetylated to Acetyl-CoA to generate Ach by the Ach-synthesizing enzyme choline acetyltransferase (ChAT). PEMT and ChAT activities were identified in cholinergic nerve terminals suggesting that these terminals could synthesize choline to be used as a source for Ach biosynthesis in neurons. It has been demonstrated that choline reaches the brain from the systemic circulation via carriers or transport proteins that are located at BBB. This suggests that changes of choline levels in the blood would then affect its levels in the brain and how much of it will be used for the synthesis of Ach by neurons. Studies also showed that choline can be de novo synthesized in the soma of neuronal cells and in presynaptic terminals (125), identifying another source for choline that will be used by neurons for structural and functional purposes. In the kennedy cycle, the endogenous synthesis of PC, a structural component of cellular membranes, includes multiple steps. Via the activity of choline kinase (CK), choline can be phosphorylated to form phosphocholine. Phosphocholine can then combine with cytidine-5'-triphosphate (CTP) to form CDP-choline. CDP-choline will then combine with diacylglycerol (DAG) to form PC via the activity of CDP-choline:1,2,diacylglycerol cholinephosphotransferase (CPT). Choline stored as membrane PC can then be used for Ach biosysnthesis as described previously.

In the synaptic cleft, Ach released by presynaptic cholinergic neurons can in turn be degraded into choline and acetic acid by Ach-destroying enzyme acetylcholinesterase (AchE). In response to this degradation, choline is released in the synaptic cleft then will be taken by presynaptic neurons via choline transporters (mainly CHT1). This reuptake via the transporters contributes to the pool of choline that will be used for the resynthesis of Ach by cholinergic neurons or will be phosphorylated back to membrane PC (126). To summarize, the main sources of choline in the brain are free choline that cross the BBB from systemic circulation, choline that is liberated from PC by the activity of phospholipases enzymes, and choline that will be picked up from the synaptic cleft by choline transporters (CHT) then will be reutilized in membrane PC after Ach degradation by AchE. So, PC and Ach are considered the main reservoirs for free choline in the brain and ChAT and AchE as the main markers of cholinergic neuronal activity or cholinergic neurotransmission.

Due to its wide ranging effects on metabolism, choline deficiency has been linked to many diseases or disorders suggesting that keeping an optimal level of this nutrient during early life and during adulthood could have positive impact on physical and mental health. Animal and human studies showed that depletion of choline from the diet during adulthood may cause organ dysfunctions such as fatty liver and liver damage due to the elevation of homocysteine in the blood (127,128). Choline deficiency was also shown to induce lymphocyte apoptosis, muscle damage and DNA breakdown or DNA damage in humans (129). It has been long recognized that premenopausal women have lower requirements for choline intake from the diet than postmenopausal women since they can synthesize choline via estrogen. The female sex hormone estrogen binds to estrogen responsive elements (ERE) along the PEMT gene and induce its expression (130) for the de novo synthesis of choline. So, depletion in choline that is induced by the reduction in the level of estrogen in post-menopausal women or by genetic polymorphism or SNPs in the hepatic PEMT gene could deprive these women of choline synthesis as they age and in some cases could increase the risk for diseases or organ damage such as cancer development (131). In the context of brain function, depletion of choline during prenatal, perinatal or postnatal life has been associated with the etiology of neural tube defects (112), possible decline of mental capabilities with age (132) and neurological disorders (15,17,20,21,120). For example, a choline-containing compound and an Ach precursor such as choline alphoscerate or glycerophosphocholine (GPC) has been suggested as a neuroprotectant agent against age-related dementia. For example, GPC has been shown to induce the release of Ach in the rat hippocampus and cause positive structural changes in that area of the brain that is associated with attention, learning and memory (114). Another choline-containing compound such as CDP-choline has been shown to delay memory deficit or decline in aged rats when this compound was supplied for a long-term period in the rat diet. As a result of this long-term supplementation, these aged rats exhibited a decrease in memory decline with time as extrapolated from their performance in memory-related tasks such as the Morris water maze (133). The positive implications of the use of CDP-choline in the aging human population are not yet explored but may warrant further investigations.

It has been well established that neurodegeneration is partly induced by a decrease in membrane PC and PEM levels (134). One of the hallmarks of Alzheimer's disease, a progressive

debilitating neurodegenerative disorder, is the gradual degeneration of cholinergic neurons and a resulting deficit in cholinergic neurotransmission in the aging brain. Since choline contributes to the structural integrity of membranes and to cholinergic neurotransmission, it may be useful to consider the possibility that choline supplementation in the diet in combination with other therapeutic interventions could boost brain activity and probably delay cognitive and memory functions or attenuate some adverse behavioral effects that are often associated with aging.

The maintenance of cellular membrane integrity has been extensively studied and has been considered as critical for cellular functions including the function of neuronal membranes including cell signaling and neurotransmission. Choline is not only a precursor of membranes' PC but also of sphingomyelin (SM), another structural component of membranes. SM supports many important physiological functions such as proper myelination of nerve cells, a process that is often accelerated during fetal life and is essential for proper neuronal signaling in the brain throughout life. It is then not surprising that abnormal levels of choline in the brain would affect the availability of this structural component and potentially could have adverse effects on brain circuitry and alter brain function across the lifespan.

5. CHOLINERGIC NEUROTRANSMISSION, MEMBRANE INTEGRITY AND NEURODEGENERATION

Deficit in cognitive functions has been associated with many neurodegenerative disorders such as AD, PD and HD. Cognitive functions require coordinated interactions of neurons in different brain regions and the maintenance of neuronal circuitries to result in normal behavior and an appropriate decision-making process at the individual level. Cognitive functions are often impaired and progressively get worse during neurodegeneration. There is mounting evidence that dysfunction and/or degeneration of the cholinergic system and dysregulation of Ach neurotransmission in specific brain regions are primarily involved in the etiology of these disorders (135). For example, patients with AD consistently showed degeneration of cholinergic neurons in the basal forebrain and decreased production of AchE and ChAT in the cerebral cortex and hippocampus (136). These two enzymes play a role in determining the pool of choline that will result from Ach degradation via AchE and how much will be reused for Ach synthesis after its reuptake by ChAT (Figure 4). Here we describe briefly what the cholinergic system is and what the physiological roles of Ach are in this system. We will also describe the location of cholinergic neurons and their projections throughout the brain and the role of phospholipids in maintaining structural and functional membrane integrity. Although the role of the cholinergic system and the role of Ach in cognitive functions are still not very clear and quite complicated, our current and future knowledge of how they function and the molecular factors or mediators that are involved in their proper functioning would reveal new information that will help to lessen the worsening of the symptoms of neurodegenerative disorders.

Ach is an important neurotransmitter in the brain that is synthesized and released by cholinergic neurons. These neurons play a role in cognitive, learning and memory functions and are vulnerable in neurodegeneration. Ach exerts its effects on cholinergic neurons function via its receptors. The two main Ach receptors are the cholinergic muscarinic Ach receptors (mAchRs) and the nicotinic Ach receptors (nAchRs). These receptors are differentially expressed in different brain regions. The binding of Ach to mAchR or nAchR differentially alters neuronal excitability and transmission. Cholinergic neurons that synthesize Ach are widely distributed in the brain with several inputs and outputs, connect different brain regions and require trophic factors such as NGF for survival and proper neurotransmission (137,138). It is then not surprising that cholinergic neurons dysfunction could adversely affect their neurotransmission in affected brain regions. Among these brain regions, the basal forebrain cholinergic neurons are probably the best understood and studied so far and have been shown to exert a modulatory action on cortical neurons function. These cholinergic neurons receive input from the prefrontal cortex, an important brain region that is involved in decision-making. In turn, forebrain and brainstem cholinergic neurons send neuronal projections into other several brain regions such as the hippocampus, amygdala, cerebral cortex and the thalamus. Interestingly, the striatum which is considered part of the limbic system, is the only brain region with a large number of cholinergic interneurons and receive dopaminergic inputs from the thalamus, the cerebral cortex and the brainstem (139). Due to the extensive connections with other neurons in different brain regions, basal forebrain cholinergic neurons are particularly vulnerable to many brain diseases or disorders especially to AD and PD. These extensive bidirectional connections of cholinergic neurons with different brain regions could explain the wide range of physiological roles of the cholinergic system in learning, attention, memory, stress and hence emotional responses, sensation and sleep regulation. Hence dysfunction of this cholinergic system would explain the wide range of symptoms that patients with neurodegenerative disorders often exhibit.

A correlative relationship between the reduction of several cortical cholinergic markers such as ChAT, AchR and Ach and progressive loss of cognition and memory suggested that the hypofunction

of the cholinergic system and altered structure and function of membranes are hallmarks in pathological aging such as AD. Dysfunction of cholinergic neurons in neurodegenerative disorders may impact negatively transmission in other neurons and compromise the activity of the brain as a whole. A hypofunction or degeneration of cholinergic neurons is a hallmark of AD or PD and probably the cause of the progressive loss of memory and cognitive impairments with aging. These observations suggest a pivotal role of the cholinergic system in the pathology of AD and PD and a potential target for treatment. The vulnerability of the cholinergic system with normal aging shows a different but consistent findings compared to neurodegenerative disorders. The normal aging brain doesn't exhibit loss of cholinergic neurons but rather a decrease in their function that is decrease in signaling or neurotransmission. In AD, cholinergic dysfunction has been associated with the accumulation of Beta-amyloid plaques and neurofibrillary tangles (NFTs) especially in the basal forebrain, hippocampus and the cerebral cortex (6). It has been suggested that beta-amyloid acts on alpha7 nAchR, the most widely expressed nAchR in the CNS, resulting in changes in the levels of NGF and phosphorylation of Tau, a microtubule-associated protein, suggesting the hypothesis that amyloid accumulation is the cause of cholinergic neurons dysfunction and loss. Another explanation is that amyloid accumulation could be the result not the cause of cholinergic neurons dysfunction in AD (140). It has been shown that hyperphosphorylated Tau leads to the formation of NFTs which progressively reduce the ability of tau to bind normally to microtubules leading to cytoskeletal instability in neuronal cells and hence neuronal death. Not only is the basal forebrain cholinergic system affected in neurodegeneration but also the cholinergic system in other brain regions was also implicated. A significant reduction in cortical cholinergic innervation has been shown in late-onset of AD and a significant decrease or loss of cholinergic functions with no cholinergic degeneration was observed in early stages of AD.

Given the critical roles of PC and SM in maintaining structural integrity of membranes, several studies demonstrated lipid membrane abnormalities in several neurodegenerative disorders including AD. The main constituent of mammalian cellular membranes is choline which is the main precursor for PC, SM or other choline-containing phospholipids. It has also been demonstrated that the largest amount of choline in the brain is found in the form of PC or SM compared to free choline. It has been established that PC biosynthesis is significantly increased during neurogenesis and in period of neurite outgrowth and neuronal differentiation to support membrane structure and function and hence neurogenesis (141). A study conducted in cultured rat sympathetic neurons showed that the inhibition of choline uptake by alkylphosphocholine correlated with a decrease in PC synthesis and impairments in axonal growth and elongation in these neurons (142). In addition to neuronal structural changes in response to choline uptake inhibition, other studies demonstrated that synapses could also be impacted by a compromised membrane integrity. Synapses are considered very crucial in neuronal connection and transmission. They include presynaptic and postsynaptic membranes that are functionally interconnected and structurally made mainly of phospholipids such as PC and SM. Embedded in these membranes are variety of ion channels and receptors that translate an external signal to a downstream intracellular signaling. This means that cellular membranes constitute an environment with many dynamic neuronal activities that need to be protected, maintained and coordinated throughout life for proper neurotransmission. Hence membrane breakdown, a structural change that is often associated with neurodegeneration, would impact membrane functioning and consequently neuronal signaling as seen in AD patients. It has been suggested that membrane breakdown during the aging process or during degeneration could occur as a compensatory mechanism to provide a large amount of free choline to be used by neurons for the synthesis of membrane and Ach. So, dietary supplementation of specific combination of nutrients including choline could preserve membrane integrity, decrease the process of membrane breakdown and attenuate synaptic loss and possibly cognitive deficits in AD.

A paradigm shift to nutritional approaches in the disease management of AD is now emerging. It has been suggested that an intervention at early stages of AD could be effective in attenuating symptoms of this disease and perhaps preventing the accumulation with time of pathological changes at the molecular level. It has also been suggested that targeting the cholinergic system by nutritional intervention could attenuate the symptoms of AD or reduce the risk for disease progression. Mounting evidence also suggests that good nutrition has beneficial effects on the aging brain including a decrease or delay in the decline of memory and cognitive functions. Recently, choline emerged as a potential nutrient that could have these beneficial effects if administered in combination with other nutrients as a preventive strategy. One study conducted in human subjects used a combination of several nutrients including choline, VitB12, VitB6 and folic acid in patients with early AD. The idea is that this combination would provide essential precursors and cofactors that would support membrane structure and function and ameliorate synaptic transmission in cholinergic neurons. This nutritional supplement resulted in memory improvements and enhancement of synaptic signaling in these AD patients (29). This suggests that nutritional approaches should be considered as a safe option in the management of

these neurodegenerative diseases and could be used as a way to attenuate the risk of progression of AD at its early stage of prognosis or detection. It should be noted here that uptake of choline from circulation into the brain decreases significantly with aging, which necessitates the necessity to increase its uptake in the older population. This reduction of choline uptake by the brain from the circulation would induce degradation of PC in neuronal membranes to keep up for choline demand that is needed for synthesis of Ach at synapses (143). The persistent reduction of choline in circulation would explain the degeneration of membrane phospholipids in these degenerative disorders and the loss of cholinergic neurons in affected brain regions that have been demonstrated in many studies. Thus, choline supplementation with other nutrients in older people or with people at early stage of AD would be of great help to increase the pool of choline uptake and its presence in the brain and its availability for Ach synthesis and the use of this later at synapses.

6. POTENTIAL NEUROPROTECTIVE EFFECTS OF CHOLINE

Choline is a fundamental nutrient that is required for normal development and functioning of the developing brain and the aging brain. Several studies suggested that maternal choline intake during critical period of brain development may accentuate cognitive functions of offspring during adulthood and attenuate age-related decline in memory (17,144–146). Other studies showed that administration of choline in the form of choline chloride or as PC improved short-term memory in some animal models and in human subjects (147,148). So, understanding the molecular mechanisms of choline and how it affects changes in gene expression and brain function at different stages of brain development could provide a potential avenue for the optimal and safe use of this nutrient with other nutrients of the one-carbon metabolism and in conjunction with other intervention methods to improve or protect mental capabilities throughout life.

The role of nutrients such as VitB12, VitB6, choline, folate and methionine in promoting health in the adult stage has been documented (149–151) and accumulating evidence points to the vital role of early life nutritional environment on the fetal epigenome and in the induction of long-term molecular and/or behavioral changes later in life (152,153). In this section, we will discuss the potential role of choline as a neuroprotectant for the fetal brain and the aging brain. We will then summarize current advances in our understanding of the potential molecular/epigenetic mechanisms that could be involved in neurodegeneration or age-related decline of memory or cognitive functions and the potential role of choline as an epigenetic modifier of the genome.

6.1. Choline and the developing brain

Fetal life is a critical developmental period that is characterized by active cellular division and differentiation in the human body. In particular, the brain exhibits a remarkable plasticity during early development and has the ability to change its wiring in response to various environmental factors including the quality of the maternal diet during pregnancy. Fetal life is characterized by the occurrence of essential physiological processes that are precisely timed such as neurogenesis, neuronal differentiation, migration and synaptogenesis. These physiological processes are governed by a set of genes that are spatially and temporally expressed and have specialized functions. This early developmental period also necessitates the availability in proper amount of essential nutrients that are methyl group donors including choline to support brain structure and brain functions that are related to learning, cognitive and memory functions throughout life. Several studies conducted in animal models demonstrated that alteration in maternal intake of choline could elicit favorable or unfavorable life-long changes on the brain and impact mental health of offspring during adulthood (154–157). It has been shown that the effects of choline on fetal gene expression are epigenetically mediated and may be induced by the exposure of the fetus microenvironment to many factors, external and internal factors. How choline could alter neuronal gene expression and function by epigenetic mechanisms?

Betaine, a choline derivative, plays a role in the remethylation of homocysteine to methionine then SAM, a major methyl-donor for methylation pathways (Figure 4). These methylation pathways include DNA methylation or histone methylation. So, changes in the level of choline and its derivatives during fetal life may modulate the activity of essential enzymes that are involved in these methylation reactions such as Dnmts or HMTs. These enzymes play a role in changing DNA or histone methylation and could alter fetal gene expression and function in such a way that these genes will be expressed or silenced. Several *in vitro* and *in vivo* studies suggested that abnormal changes in prenatal, perinatal or postnatal choline levels may cause long-lasting structural, functional or behavioral changes at the organismal level that manifest themselves later on in life. For example, a low maternal intake of choline has been shown to cause neural tube defect in humans and in rodents (112,158) and to negatively impact neuronal migration, survival and differentiation (159,160). Choline deficiency reduced neural progenitor cell (NPC) proliferation and migration in the fetal hippocampus of rodents (161). Choline deficiency during prenatal or early postnatal life also caused impairments in certain aspects of memory formation in specific memory-related tasks whereas its supplementation from

embryonic days E11-E17 reversed these effects by inducing NPC proliferation in rat fetal hippocampus and improving rat memory or cognitive functions with age (146,162–164). Other studies demonstrated the positive effects of prenatal and perinatal choline on mental performance later in life. For example, prenatal supplementation of choline chloride in the maternal diet resulted in long-term improvements of offspring in many memory-related tasks and in some cases these rats showed a decrement in age-related memory decline (146,164,165).

How does choline induce changes in gene expression by epigenetic mechanisms? Alteration in choline levels in the brain has been shown to cause global or gene-specific histone methylation (157,166) and/or DNA methylation (53,167) in a tissue-specific manner. For example, *In vivo* studies showed that choline deficiency at E17 resulted in a decrease in the expression of G9a histone-methyltransferase and its associated repressive marks H3K9me1 and H3K9me2 in the subventricular zone and the ventricular zone of the hippocampus with no changes in global levels of these repressive histone marks in the whole mouse fetal brain. These repressive histone marks correlated with a decrease in the binding of the repressor element 1-silencing transcription factor (REST) on the repressor element-1 (RE1) site of the calbindin gene Calb1 promoter. This decrease in the binding of the repressor on Calb1 promoter created an environment that is conducive for transcription upstream of the RE1 site of *Calb1*gene and increased its expression in NPC. It has been suggested that there is a correlative relationship between DNA methylation and the predominance of the methylated repressive mark H3K9 in causing gene repression (168). Perhaps this decrease in the occurrence of these repressive histone marks could have resulted in a decrease in the Calb1 gene promoter methylation. They found that choline deficiency increased the methylation of only one CpG site along the Calb1 gene promoter in cultured NPC with no changes in total methylation of the CpG island of this gene (51). Collectively, these data suggest that an alteration in choline levels during fetal life may have altered hippocampal neurogenesis by causing epigenetic changes and altering the expression of key genes that are related to neurogenesis in fetal brain. These epigenetic changes that have been induced by choline deficiency in the hippocampus could alter memory functions later in life and accelerate the decline of memory functions with aging.

Prenatal choline supplementation has been shown to alter brain plasticity that is related to memory and cognitive functions throughout life (169). Memory functions most often involve a series of complex molecular changes that modulate gene expression in the brain. While these molecular changes could be induced by specific transcription factors or intracellular signaling factors, considerable evidence suggests that choline, as an epigenetic modifier, could also modulate gene expression and brain function in response to neuronal activity and environmental factors (51,53,170). At the gene level, microarray experiments demonstrated that prenatal choline supplementation caused remarkable changes in the expression of memory-related genes in both the hippocampus and the cerebral cortex (171), suggesting a correlative relationship between the availability of choline during early life and the induction of these molecular changes in key memory-related genes. At the behavioral level, prenatal choline supplementation enhanced neurogenesis in the dentate gyrus of rat hippocampus, the brain memory center, and elevated the levels of the brain-derived neurotrophic factor (BDNF). Other memory-related genes that were also altered by prenatal choline supplementation is the insulin-like growth factor IGF-1. IGF-1 levels were elevated in the rat brain and differentially altered the performance of these rats in specific memory-related tasks (172). Prenatal choline also elevated IGF-II and its receptor (IGFII-R) in the rat hippocampus and the frontal cortex, suggesting a vital role of choline in regulating cholinergic functions in these two main brain regions that regulate memory and executive functions (116).

Prenatal choline supplementation did not only cause molecular and behavioral changes but also enduring structural changes by increasing the size of cell bodies of basal forebrain cholinergic neurons that are projecting to the hippocampus (173), inducing Ach release by these neurons (174) and reducing the activity of AchE in the hippocampus (175). Prenatal choline supplementation resulted in long-term increase in dendritic arborization of hippocampal CA1 pyramidal neurons of rats (176) suggesting an increase in neuronal connectivity in that brain region. At the circuitry level, prenatal choline supplementation in rats increased N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission during adulthood (177). Prenatal choline chloride also increased significantly the levels of NGF, an essential factor for cholinergic neurons survival and neurotransmission, in the frontal cortex and the hippocampus of adult rats (178), all are physiological processes that affect neuronal survival and neuronal connectivity in the memory region of the rat brain.

The long-term effects of the use of other nutrients in conjunction with choline on the brain were also studied. Methyl-donor deficiency including folate, methionine and choline caused molecular changes in the hippocampus of adolescent mice resulting in behavioral changes that are linked to learning and memory (179). Collectively, these studies suggest that choline supplementation during early life, a critical period for normal brain development, may have long-

term beneficial effects on cognitive functions and memory processes in the brain later on in life.

6.2. Choline and the aging brain

The prevalence of neurodegenerative disorders is on the rise worldwide which imposes a huge burden on society and afflicted individuals. AD is an example of a debilitating neurodegenerative disease that is multifactorial, polygenic with wide range of symptoms. Dysregulation in the expression of several genes has been identified in the AD brains. Emerging evidence suggests that this dysregulation in gene expression is probably caused by genetic factors but also could be induced by epigenetic mechanisms in response to consistent exposure to environmental factors throughout life (151,180,181). Depending on the timing and the duration of exposure to these factors, changes in the expression of many genes in the brain may occur later in life resulting in multifaceted behavioral changes including decline in cognitive and memory functions. An increasing attention has been lately devoted into understanding the underlying molecular mechanisms of cognitive and memory dysfunctions with aging in the goal of identifying effective treatments to promote brain longevity and increase individual's productivity. Identifying these mechanisms has been proven to be quite challenging and so far no effective treatment has been proven to attenuate the occurrence of this neurodegenerative disorder. This fact would require from the scientific community to redirect the effort toward spotting changes in the expression of a network of genes in the brain or changes in their epigenetic landscape. The onset and/or progression of this disorder are heterogeneous and may be induced not only by the individual's genetic make-up but also exposure to environmental factors including nutrition and lifestyle (182). A holistic approach is needed in the near future to identify specific epigenetic changes in the brain of individuals with neurodegenerative disorders then develop individualized treatment that include drugs that target these specific epigenetic changes. Not only we do have to target these specific epigenetic changes but also supplement afflicted patients as part of the treatment with specific nutrients such as choline, VitB12, VitB6 and folic acid that have been proven to improve mental functioning.

Several studies showed that aged-mice exhibited positive behavioral changes related to learning and memory when placed on a choline-rich diet (183). What are the epigenetic changes that have been identified in Alzheimer's diseases? Earlier studies showed that mutations in the presenilin gene and the amyloid precursor transmembrane protein (APP) gene (184) are so far consistent molecular changes in patients with Alzheimer's disease. Recently, it has been proposed that alteration in memory formation may be linked to epigenetic mechanisms such as changes in DNA methylation (185). For example, hypomethylation of the APP gene promoter (186) has been identified in many AD patients. Changes in histone marks (187) or changes in the levels of specific types of histone deacetylases (HDACs) including HDAC2 and Sirtuin1 (188,189) were also detected in the hippocampus of postmortem AD patients. These findings suggest that this neurodegenerative disorder could also be an epigenetic disorder. This necessitates perhaps the need to time these epigenetic changes during the progression of this debilitating disease in the goal of identifying therapeutic intervention that temporally and spatially target these epigenetic changes to delay or attenuate the decline in brain cognitive and memory functions.

The link between the quality and quantity of nutrients, that are often consumed throughout life, correlates with mental activities such as learning, memory and cognitive functions (151,190,191) suggesting that the quality of an individual's diet and lifestyle may play a role in affecting mental capabilities in beneficial or detrimental ways. Several dietary components or natural products such as choline were identified as neuroprotectants and should be taken in optimal amount for proper brain function (53,192) as a decrease in choline levels has been consistently reported with aging in some individuals (193). Few human studies also showed that higher choline intake positively correlated with better performance in memory tests (194). Human studies showed that a nutrient supplement "SOUVENAID" that consists of uridine, omega3-fatty acids (DHA) and choline showed an improvement in memory scores in patients with early AD and an increase in brain synapses and neuronal communication in different brain regions. This study suggests that the combination of these nutrients may have contributed to an increase in brain PC synthesis and hence membrane integrity and positively impacted the structural and functional integrity of presynaptic and postsynaptic membranes, sites where most synapses or synaptogenesis occur in the brain (195–197).

A hypofunction of cholinergic neurons in the cerebral cortex and in other brain areas also indicate that alteration in choline levels and or its metabolites in the brain may be linked to the etiology of AD (198). A decrease in Ach synthesis have been shown to correlate with significant cognitive impairments in AD (199,200). A dysfunction of glutamatergic signaling that is mediated by NMDA receptors was identified as another plausible mechanism that participates in the etiology of AD (201,202). Collectively, these findings suggest that normal level of choline in the diet and in the brain may attenuate mental decline with age.

Membrane remodeling has been also acknowledged as a potential mechanism in the etiology of AD. Abnormal change in membrane structure and

function, a hallmark of many neurodegenerative disorders (203), is now accepted as a physiological process that could negatively impact cellular viability and neurotransmission in different areas in the brain. Thus, attenuation of this process of membrane breakdown may attenuate some of the symptoms that are associated with some neurodegenerative disorders. In particular, changes of choline-containing phospholipids have been suggested to play a role in membrane breakdown and affect brain function. Postmortem studies demonstrated a reduction in the levels of membrane phospholipids in aging and demented human brains (204,205). Earlier studies suggested that PC breakdown or a decrease in the levels of choline metabolites may contribute to the degeneration of cholinergic neurons in AD (126,206). A reduction in the activity of ChAT in cholinergic neurons was also detected in several brain regions of AD patients (120,207,208) whereas inhibitors of AchE have been proven to have beneficial effects. Additionally, three cortical brain regions of postmortem brain of AD patients showed a significant depletion in the levels of two main components of the phospholipid bilayer such as PC and PEM and a correlative decrease in the levels of their precursors such as choline and ethanolamine (134). Taking into account these findings, choline could be a nutritional therapeutic approach that could mitigate the severity of AD symptoms or possibly the symptoms that are associated with other neurodegenerative disorders.

Changes in choline levels have been also linked to other brain insults that may accelerate the development and/or progression of some neurodegenerative disorders. For example, brain choline levels were altered under different pathological conditions including hypoxia (209), ischemia (210,211) and seizures (212). It has been shown in several studies that the development of seizure and its severity could have long-term adverse effects on brain function including changes in behavior, learning, memory and cognitive functions (213,214). Recent study demonstrated that oral administration of choline to ischemic rats increased CA1 hippocampal neuron survival (215). Prenatal choline supplementation attenuated status epilepticus-induced memory impairment in adult rodents (23,25,216). Whether these findings in animal models could be extrapolated to human conditions remain to be determined. Collectively, these studies suggest that nutritional intervention such as choline supplementation may attenuate the vulnerability of cognitive and memory functions to various brain insults later in life.

7. CONCLUSION

Our understanding of the mechanisms that are involved in many neurodegenerative disorders is incrementally increasing. The advancement of molecular techniques and neuroimaging made it possible to identify key molecular targets that are involved in specific type of neurodegeneration. It also reveals structural modifications and changes in circuitry in specific brain regions that are affected by these disorders. The complexity of neurodegenerative disorders made it very difficult till now to develop an effective treatment to attenuate the symptoms of these disorders or be able to intervene at an early stage of diagnosis. Emerging evidence reveals that the complexity and the severity of these disorders are due to a combination of genetic factors and environmental factors. Environmental factors have been shown to interact with our genes and modulate their functions. Diet or nutrients could impact our health in a positive and negative manner across the lifespan. Selected human studies showed that specific nutrients if applied during early stage of some of these neurodegenerative disorders could attenuate the progression of these disorders and protect cognitive functions of the brain with aging. Among these nutrients are those that participate in the folate-mediated one-carbon metabolism such as folate, choline, betaine, methionine, VitB12 and VitB6. Although the potential positive effects of these nutrients on mental health is still not very clear especially in humans, several studies conducted in animal models proved their long-lasting effects in promoting and protecting cognitive functions. Future research into neurodegeneration should emphasize the exploration of a personalized treatment that requires to target specific epigenetic targets that are involved in neurodegeneration and explore the effects of nutrients supplementation at an early age and possibly later in life on the amelioration of brain function.

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Abbreviations: Acetylcholine: Ach, Acetylcholine esterase: AchE, Acetylcholine receptor: AchR, S-adenosylhomocysteine: SAH, S-adenosylmethionine : SAM, Alzheimer disease: AD, Amyloid precursor protein: APP, Arginine:R, Brain derived neurotrophic factor: BDNF, Choline acetyltransferase : CHAT, Choline transporter: CHT, DNA methyltransferase: DNMT, Embryonic day: E, Histone acetyltransferase: HAT, Histone deacetylase: HDAC, Histone deacetylase inhibitor: HDACi, Histone methyltransferase: HMT, Huntington disease: HD, Hypothalamic-pituitary-adrenal : HPA, Insulin-like growth factor: IGF, Lysine:K, Methyl :CH3, 5-methylcytosine: 5-mC, Methyl-binding domain: MBD, Methyl-CpG- binding protein 2: MeCP2, 5-methyltetrahydrofolate: 5MTHF, Methyltetrahydrofolate reductase: MTHFR, microRNA: miR, miRNA recognition element: MRE, Muscarinic acetylcholine

receptor: mAchR, Neural progenitor cell : NPC, Nicotinic acetylcholine receptor: nAchR, N-methyl-D-aspartate: NMDA, Nucleotide: nt, Parkinson's disease: PD, Phosphotidylcholine: PC, Phosphotidylethanolamine : PEM, Post-translational modification: PTM, Repressor element-1: RE1, RNA-induced silencing complex: RISC, Serine:S, Single nucleotide polymorphism: SNP, Tetrahydrofolate: THF, 3'-untranslated region: 3'UTR

Key Words: Choline, Environment, Epigenetics, Neurodegeneration, Nutrition, Review

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