How and When Environmental Agents and Dietary Factors Affect the Course of Alzheimer’s Disease: The “LEARn” Model (Latent Early-Life Associated Regulation) May Explain the Triggering of AD

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Abstract: Alzheimer’s disease (AD) is currently the most prominent form of dementia among the elderly. Although AD manifests in late adult life, it is not clear when the disease actually starts and how long the neuropathological processes take to develop AD. The major unresolved question is the timing and the nature of triggering leading to AD. Is it an early or developmental and/or late phenomenon and what are the factors that trigger the cascade of pathobiochemical processes? To explain the etiology of AD one should consider the neuropathological features, such as neuronal cell death, tangles, and amyloid plaque, and environmental factors associated with AD, such as diet, toxicological exposure, and hormonal factors. Current dominant theories of AD etiology are “protein–only”, they attribute the cause of the disease directly to the activities of associated proteins once they have been produced; the major limitation is that protein aggregations occur “late in the game”. Development and progression of AD has not been explained by protein–only models. In view of this limitation, we propose a “Latent Early-Life Associated Regulation” (LEARn) model, which postulates a latent expression of specific genes triggered at the developmental stage. According to this model, environmental agents (e.g., heavy metals), intrinsic factors (e.g., cytokines), and dietary factors (e.g., cholesterol) perturb gene regulation in a long–term fashion, beginning at early developmental stages; however, these perturbations do not have pathological results until significantly later in life. For example, such actions would perturb APP gene regulation at very early stage via its transcriptional machinery, leading to delayed overexpression of APP and subsequently of Aβ deposition. This model operates on the regulatory region (promoter) of the gene and by the effect of methylation at certain sites within the promoter of specific genes. Promoters tend to have both positive and negative regulatory elements, and promoter activity can be altered by changes in the primary DNA sequence and by epigenetic changes through mechanisms such as DNA methylation at CpG dinucleotides or oxidation of guanosine residues. The basis of the LEARn model is that environmental factors, including metals and dietary factors, operate by interfering the interaction of methylated CpG clusters with binding proteins, such as MeCP2 and SP1. The LEARn model may explain the etiology of AD and other neuropsychiatric and developmental disorders.

Keywords: Aging, cholesterol, dementia, development, diet, epigenetics, LEARn, metal, methylation, promoter, psychiatric disorders, gene regulation, risk factors.

PREVALENCE AND SOCIAL COST OF ALZHEIMER’S DISEASE

Alzheimer’s disease (AD) is currently the most prominent form of dementia among the elderly (up to 76%) in Western societies [1]. Major symptoms include severe loss of memory, cognition, reasoning, and other intellectual abilities. In 2003, an estimated 4.5 million people in the USA had the disorder [2] and it was the 8th most prevalent cause of death for that year [3]. Prevalence of the disease increases by age—approximately 1% at age 60–64, doubling every five years up to 40% at age 85 years and over [1]. AD in the USA has been projected to increase between 11 and 16 million by 2025–2050 [2, 4]. Worldwide estimations of all forms of age–related dementia (subjects age 60+) are between 24 and 28 million people [5, 6]. In addition to those directly afflicted, AD carries an added burden to caregivers that is related economically and psychologically to severity of the disease [7–9] and wider social burden is likely greater, given that one in ten US residents report a relative with AD and one in three report acquaintance with an AD patient [10].

HALLMARKS OF AD: ROLE OF β–AMYLOID AND τ PROTEINS

Neuropathologically, AD is characterized by neurodegeneration and accumulation of proteinaceous aggregates, specifically intracellular hyperphosphorylated microtubule associated protein τ “tangles” and extracellular plaques formed predominantly of the amyloid–β peptide (Aβ) [11, 12], itself derived from the β–amyloid precursor protein (APP). These two aggregates have led to the two currently–dominant models in the field, specifically the “amyloid hypothesis” and the “τ hypothesis”. The amyloid hypothesis posits the neurotoxicity of Aβ dimers and oligomers and/or damage caused by Aβ plaque aggregation as the primary cause of AD symptoms [13–15]. The τ hypothesis states that
aggregation of hyperphosphorylated \( \tau \) leads to neuronal cell death and resulting behavioral symptoms and neuropathology (Fig. 1). A\( \beta \) aggregation would be a result of cellular damage imposed by \( \tau \) aggregation [12, 16, 17]. In addition, alternative hypotheses exist based on systems–analysis principles, such as suggesting failure of APP metabolism/clearance that would create a “traffic jam” in membrane protein turnover, ultimately leading to multiple system failure [18]. Moreover, there is some question regarding whether or not these AD–associated aggregates are an immediate cause of or actually a protective response to neurodegeneration [19]. Beyond A\( \beta \) and \( \tau \) pathways, cholinergic dysfunction and oxidative stress are also implicated in AD (Fig. 1).

GENETICS AND SPORADIC NATURE OF AD

While a minority (approximately 5%) of AD is attributable to familial AD (FAD) mutations in the coding sequences of AD–associated genes, such as APP and presenilin 1 (PSEN1) [20], this form of AD does not explain the more common sporadic late–onset AD (LOAD). Known risk factors for sporadic LOAD include age [1, 21] limited education [21-23], head trauma [24], dietary cholesterol [25], the APOE\( ^4 \) genotype [26, 27], and further associations with additional proteins such as the insulin degrading enzyme (IDE) [28-30], \( \alpha_2 \)--macroglobulin [31] and endothelin converting enzyme 2 (ECE2) [32]. In addition, AD risk has been associated with promoter polymorphisms in the APOE [33-35] and APP genes [36]. Oxidative stress in the brain [37-43] and inflammatory factors such as NF–\( \kappa \)--B [44-49] are likewise linked to AD. Clinical and mechanistic research have shown a protective effect for non–steroidal anti–inflammatory (NSAID) drugs [50-56]. In addition, acute exposure to metals such as copper and lead have been shown to perturb expression of the APP gene [57-65]. However, none of the current etiological models have proven sufficient to explain the sporadic nature of AD and the “incomplete” effects of known risk factors. Therefore, it is likely that AD pathology is a combination of pathways and effects (Fig. 1).

THE APP PROCESSING PATHWAY: AMYLOIDOGENIC AND NON–AMYLOIDOGENIC PATHWAYS

In all forms of AD, accumulation of extracellular amyloid plaque consisting primarily of the A\( \beta \) peptide is the definitive diagnostic criterion [66, 67]. The A\( \beta \) peptide is derived from APP, a 115 kDa trans–membrane protein that is processed by the 
secretase pathway to produce soluble extracellular APP (sAPP) and various smaller peptides, depending upon the individual processing pathway. In the non–amyloidogenic pathway, APP is cleaved by the \( \alpha \)--secretase ADAM17, (TACE), generating the neuroprotective sAPP\( \alpha \). The remaining fragment is cleaved by the \( \gamma \)--secretase complex, which includes PSEN1, to produce the P3 and CTF\( \gamma \) fragments. In the amyloidogenic (AD–associated pathway), APP is cleaved by the \( \beta \)--secretase, \( \beta \)--amyloid cleaving enzyme (BACE1), generating sAPP\( \beta \). The remaining short peptide is cleaved by \( \gamma \)--secretase to produce CTF\( \gamma \) and A\( \beta \). Thus, it is cleavage of APP by \( \beta \)--secretase (BACE1) that specifically determines the production of A\( \beta \) from the APP precursor [68], making this the rate–limiting step in the production of A\( \beta \) from APP.

UNRESOLVED QUESTIONS IN THE ETIOLOGY OF AD

Although AD manifests late in adult life, it is not clear when the disease actually starts and how long the neuropa-

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**Fig. (1). Factors contributing to Alzheimer’s disease.** Schematic diagram showing that Alzheimer’s disease is believed to be caused by a cascade of biochemical events, such as increased amyloid \( \beta \) deposition and accumulation of hyperphosphorylated \( \tau \). Other AD hallmarks include reduced cholinergic markers, increased oxidative stress, and several environmental and epigenetic factors. How and when these factors trigger the disease are not clearly understood, and the LEARn model is proposed (Fig. 3) to address these questions.
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neurological processes take to develop to what is recognized as AD. Therefore, the major unresolved question is the timing and nature of triggering that leads to the AD pathological pathway(s). Regarding the “timing” issue, is it an early/developmental or late phenomenon? Another unanswered question is what factors, if any, are able to trigger the cascade of pathobiological processes of the disease.

CRITERIA FOR A UNIFYING HYPOTHESIS OF AD ETIOLOGY

A unifying hypothesis to explain the etiology of AD should take into account not only neuropathological features, such as neuronal cell death, tangles, and amyloid plaque, but also the various environmental factors associated with AD, such as diet, toxicological exposure, and hormonal factors. In the amyloid hypothesis, overproduction of Aβ peptides results in formation of neurotoxic oligomerized Aβ protofibrils, which further aggregate in amyloid deposition (plaques). Reaction to the neurotoxicity of Aβ may likewise contribute directly to the hyperphosphorylation of τ and consequent τ tangles [69]. Environmental agents, including drugs, diet, toxicological exposure, and hormonal factors start operating on gene expression at very early stages of development. Such actions would perturb APP gene regulation via its transcriptional machinery, leading to delayed overexpression of APP and, subsequently, of Aβ peptides.

LIMITATIONS OF THE CURRENT “PROTEIN–ONLY” HYPOTHESIS

Current dominant theories of AD etiology are “protein–only”, they attribute the cause of the disease directly to the activities of associated proteins once they have been produced. The fundamental limit of these theories is that protein aggregations occur “late in the game”. Development and progression of AD has not been explained by protein–only models, nor has it been explained by immediate extensions of these models that look for acute regulatory perturbation of genes such as APP and BACE1.

PROPOSAL OF A UNIFYING LEARn MODEL TO EXPLAIN THE INTERACTION OF ENVIRONMENT AND GENES

In response, we propose a “Latent Early-Life Associated Regulation” (LEARn) model to explain the etiology of AD, and possibly other neuropsychiatric and developmental disorders. The LEARn model states that environmental agents (e.g., heavy metals), intrinsic factors (e.g., cytokines), and dietary factors (e.g., folate and cholesterol) perturb gene regulation in a long–term fashion, beginning at early developmental stages, but that these perturbations do not have pathological results until significantly later in life. Similar hypotheses were developed in the 1980s by Barker and colleagues [70]. However, that model is predicated upon low birth weight and rapid childhood weight gain. While later health effects of such conditions are indisputable, the LEARn model is based on the regulatory structure common to eukaryotic genes (Fig. 2) and the effect of methylation at certain specific sites within the promoter (regulatory) region of specific genes (Fig. 3). In essence, it complements the classic “Barker model” of fetal origins of adult disease.

THE LEARn MODEL OPERATES ON GENES’ REGULATORY REGIONS AND AT THE EPIGENETIC LEVEL

A given eukaryotic gene consists of more than its protein–coding sequence. The 5′–flanking regulatory region is of particular use to the LEARn model. The APP gene 5′–flanking region (Fig. 2) serves as an example of a promoter that may be subject to the LEARn model. The reference point for all 5′–flanking regions is the “+1” transcription start site (TSS). The region between the TSS and the first ATG codon is the 5′–untranslated region (UTR). A survey of 2,312 human 5–UTR sequences produced a range of 3bp to 9150bp in length, with an average length of 212bp ± 377 bp (standard deviation) [71]. 5′–UTR sequences are known to have regulatory functions at both transcriptional and translational levels. The promoter sequence, upstream of the TSS, functions in transcription, and active elements can be found

![Fig. (2). The APP 5′–flanking regulatory region.](image)
multiple kilobases away from the +1. Promoters tend to have both positive and negative regulatory elements, and the activity of a promoter can be altered by changes in the primary DNA sequence and by epigenetic changes through mechanisms such as DNA methylation, which would result in hypomethylation. These changes alter the affinity for an AD-associated gene’s promoter to transcription factors such as MeCP2 (hypomethyl derepression) or SP1 (hypomethyl activation). Increased transcription of the gene results in greater production of associated genes and resultant Aβ products, which contributes to neurodegeneration, both directly and through hyperphosphorylation of microtubule associated protein τ. The net result would be AD.

Promoter Methylation Serves as a “Control Switch”

Human DNA is most commonly modified by DNA methylation, which involves the addition of a methyl group to cytosine residues at CpG dinucleotides [72]. This reaction is catalyzed by DNA methyltransferase (DNMT) enzymes. In the DNA sequence, CpG dinucleotides are found in clusters called CpG islands. Notably, they are unevenly distributed across the human genome, with approximately 30,000 CpG islands in the genome, out of which 50–60% are found within the promoter regions of genes. However, in normal tissues, CpG islands are primarily unmethylated, and the aberrant methylation of CpG islands is most likely related to disease.

How does methylation regulate gene expression? Current research suggests that MeCP2, which is a member of a family of proteins that selectively recognizes methylated CpGs, is a primary factor. The binding of such proteins to DNA results in an altered chromatin structure, which subsequently prevents the binding of the transcription machinery, and thus precludes gene expression. Abnormal methylation causes transcriptional disruption of numerous genes, leading to tumor growth and development. Studies of DNA methylation in cancer have uncovered new potential targets for the diagnosis, prognosis, and ultimate treatment of human cancer. As a rule of thumb, hypomethylation in the promoter region leads to increased gene expression, whereas hypermethylation results in decreased gene expression.

It has been shown that maternal grooming changed the methylation pattern and expression of the glucocorticoid receptor in the hippocampus in rat offspring, resulting in permanent changes in their stress response [73]. Recently, Lillycrop et al. restricted the maternal diet and studied the expression of the glucocorticoid receptor (GR) and the per-
oxisomal proliferator-activated receptor (PPAR) in the offspring after weaning. They found a decrease in the methylation of these genes that was consistent with their elevated mRNA expression. In addition to behavioral and nutritional imbalances, chemical exposure can also interfere with the status of DNA methylation [74]. One way by which environmental agents or occupational exposure could interfere with DNA methylation is by disrupting the enzymes that conduct such reactions. Poirier and Vlasova reported that the addition of cadmium (Cd) to hepatic nuclear extracts inhibited DNMT [75]. More recently, Takiguchi et al. reported that subchronic exposure to Cd inhibited DNMT activity in cultured cells, while chronic exposure enhanced the activity of DNMT [76]. They also found that the level of DNA methylation was similarly changed and suggested that the action of Cd on DNA methylation may be responsible for its carcinogenic properties.

The LEARn model rests on the notion that environmental factors, including metals and dietary factors, may operate by interfering with the interaction of methylated CpG clusters with binding proteins, such as MeCP2 and S1P. Thus, the LEARn model uses a “promoter methylation” mechanism as a switch to control gene expression. (Fig. 3)

ADVANTAGES OF THE LEARn MODEL TO EXPLAIN THE ETIOLOGY OF AD, WITH SOME POTENTIAL PITFALLS

The field of AD research currently experiences a split between “genetic” and “environmental” schools. A specific gene sequence (coding or promoter) might entice greater vulnerability to (or protection from) environmental triggers but need not automatically lead to disease in the presence of these triggers. Triggering would function only within a specific developmental window. The primary positive evidence in favor of this model is that age is the greatest risk factor for AD [37, 77]. In addition, the sporadic, “circumstantial”, nature of LOAD genetic risk factors such as APOE genotype and the accumulation of greater oxidative damage in AD brains [37] suggest significant environmental input.

The LEARn model should also be discussed in the context of the risk posed by the APOE4 allele. For example, in the case of APOE4, a Nigerian Yoruba population demonstrated lack of association between AD and the APOE4 allele, while a simultaneously–studied African–American population did demonstrate this association [78, 79]. This strongly suggests that even markers that are well known to be “genetic” may be, instead, significantly modulated by environmental factors endemic to the conditions of the majority of currently–common study populations, thus masking a LEARn–type etiology.

THE LEARn MODEL VS. FAD TYPE AD

In addition to potential challenges to the LEARn model posed by the APOE4 allele, currently well–characterized types of FAD could call it into question. These forms of AD are determined by mutations in the coding sequences of the APP or PSEN1 genes and result in pathology regardless of possible regulatory modification. However, these forms of AD represent a minority of the patient population. Up to 95% of AD cases do not involve amino acid mutations in the APP or PSEN1 proteins. The LEARn model does not purport to explain FAD but those cases that cannot be explained by the FAD model.

BASIS OF THE LEARn MODEL: INTERACTION OF A GENE’S PROMOTER WITH ENVIRONMENTAL FACTORS

Study of the gene regulatory regions that would be affected by LEARn requires characterization of the 5′–flanking sequences of several AD–associated and putative AD–associated genes (Table 1). These would include (but not be restricted to) genes such as APP, BACE1, BACE2, τ, APOE, insulin degrading enzyme (IDE), and endothelin converting enzyme 2 (ECE2). Of these, the promoter sequences have been cloned and characterized for APP [36, 80-90], BACE1 [91-94], BACE2 [97], APOE [33, 35, 99-104], and τ [105-107], while the IDE and ECE2 promoters are, as yet, not examined in as much detail.

USE OF CELL CULTURE MODELS

Study of appropriate promoter sequences may and has been carried out in cell culture by means of surveying reporter clone deletion series for genes such as APP [86, 89], BACE1 [91, 92], BACE2 [95], τ [105-107], and APOE [101, 103]. Cell culture has also proven valuable for elucidation of specific transcription factor sites within promoters including those for APP [108, 109], APOE [104], and BACE1 [91], for induction studies with various cytokines, drugs, and environmental factors [56, 63, 109, 110], and examination of oxidative stress [43, 59, 111, 112].

THE LEARn MODEL EXPLAINS DEVELOPMENTAL TRIGGERING AND LATENT EXPRESSION OF THE APP GENE

The APP protein and Aβ peptide appear in healthy individuals. Studies from knockout animals have indicated that APP has necessary functions, although there is redundancy with APP protein family members [58, 113]. In addition, evidence exists that Aβ may function as a transcription factor [114]. Therefore, what would trigger APP and Aβ peptides to be overproduced in sporadic cases of AD? More specifically, i) When does this triggering mechanism kick in? ii) What is the trigger's site (or sites) of action with the APP metabolic machinery. iii) Is the trigger within the gene, a property of the protein, of aggregation, and/or of processing enzymes? iv) Finally, how is this triggering maintained? Any complete model of AD etiology must answer these questions. The LEARn model proposes that the initial APP triggering mechanism activates early in life, at developmental stages. Sites of action would be within the promoter of APP and associated genes. The trigger is primarily a property of genetic regulation. It is maintained through epigenetic means, such as DNA methylation.

MECHANISMS OF THE LEARn MODEL: ENVIRONMENTALLY–INDUCED INHIBITION OF EARLY METHYLATION

Recently, experiments have been done to address the question of environmental effects on delayed expression patterns of genes in the context of AD. Rat pups were ex-
posed to lead (Pb) acetate from birth through weaning. Levels of AD–related gene expression such as APP and BACE1, transcription factors such as SP1, and oxidative repair enzymes such as SOD1 were measured at senescence (20 months). In addition, levels of \( -\), \( -\), and \( -\) secretase activity were measured at senescence. This was done in rats that were exposed to i) 200ppm Pb–acetate early in life but not at senescence, ii) 200ppm at senescence, and iii) no Pb–acetate exposure. Early–exposed rats showed elevated levels of SP1 and APP when compared both to late–exposed and non–exposed rats. Secretase activities were not altered among the groups. In addition, oxidative DNA damage (as measured by Oxo–d8–GTP) was greater in early–exposed rats than in other groups. However, the activities of enzymes such as superoxide dismutase1 (SOD1) and SOD2 were not altered among groups [57]. Comparison of the cumulative potential methylation CpG dinucleotides 2 kilobases (kb) upstream of the transcription start sites indicated that the proximal promoter regions surveyed by Bolin, et al suggested a difference in methylation site density centered around \( \pm 100 [57]. \n
**Table 1. AD–Associated Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
<th>Chr.</th>
<th>Function or connection</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>amyloid precursor protein</td>
<td>21</td>
<td>precursor to A(\beta)</td>
</tr>
<tr>
<td>MAPT</td>
<td>microtubule associated protein (\tau)</td>
<td>17</td>
<td>hyperphosphorylated (\tau) produces AD &quot;(\tau) tangles&quot;</td>
</tr>
<tr>
<td>ADAM17</td>
<td>TACE</td>
<td>2</td>
<td>active component the (\alpha)–secretase complex that cleaves APP in the non–amyloidogenic pathway.</td>
</tr>
<tr>
<td>ECE2</td>
<td>endothelin converting enzyme 2</td>
<td>3</td>
<td>potentially degrades A(\beta) and amyloid plaque.</td>
</tr>
<tr>
<td>NFKB1</td>
<td>NF-(\kappa)-B</td>
<td>4</td>
<td>transcription factor that regulates APP response to injury and inflammation.</td>
</tr>
<tr>
<td>SNCA</td>
<td>(\alpha)–synuclein</td>
<td>4</td>
<td>found in amyloid plaque</td>
</tr>
<tr>
<td>ADAM9</td>
<td>ADAM metallopeptidase domain 10</td>
<td>8</td>
<td>part of the (\alpha)–secretase complex that cleaves APP in the non–amyloidogenic pathway.</td>
</tr>
<tr>
<td>CTNNA3</td>
<td>(\alpha)–T–catenin</td>
<td>10</td>
<td>associated with late–onset AD.</td>
</tr>
<tr>
<td>IDE</td>
<td>insulin degrading enzyme</td>
<td>10</td>
<td>potentially degrades A(\beta) and amyloid plaque.</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
<td>10</td>
<td>alters (\tau) phosphorylation</td>
</tr>
<tr>
<td>TFAM</td>
<td>Transcription factor 6-like 2</td>
<td>10</td>
<td>associated with late–onset AD.</td>
</tr>
<tr>
<td>BACE1</td>
<td>(\beta)–secretase</td>
<td>11</td>
<td>cleaves APP in the amyloidogenic pathway</td>
</tr>
<tr>
<td>PSEN1</td>
<td>presenilin 1</td>
<td>14</td>
<td>necessary for (\gamma)–secretase activity, cleavage in both amyloidogenic and non–amyloidogenic pathways.</td>
</tr>
<tr>
<td>ADAM10</td>
<td>ADAM metallopeptidase domain 10</td>
<td>15</td>
<td>part of the (\alpha)–secretase complex that cleaves APP in the non–amyloidogenic pathway.</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme 1</td>
<td>17</td>
<td>potentially degrades A(\beta) and amyloid plaque.</td>
</tr>
<tr>
<td>APOE</td>
<td>apolipoprotein E</td>
<td>19</td>
<td>cholesterol processing; (e4) allele is a risk factor for AD in industrialized societies.</td>
</tr>
<tr>
<td>BACE2</td>
<td>(\beta)–site APP-cleaving enzyme 2</td>
<td>21</td>
<td>may cleave APP in alternate non–amyloidogenic pathway</td>
</tr>
</tbody>
</table>

SPI1, A CRITICAL TRANSCRIPTION FACTOR IN THE REGULATION OF AMYLOIDOGENESIS

When comparing early–exposed vs. late–exposed and non–exposed rats, the SP1 transcription factor had a specific mRNA level increase [65]. This was accompanied by an increase in measured levels of APP and A\(\beta\). In addition, SP1 has been shown to upregulate the human BACE1 gene [91] and to be active in regulation of both human [115] and rat [116] APP genes. SP1 is a ubiquitous transcription factor that operates in multiple tissue types. It has been shown, along with SP3, to be elevated in cortical neurons that have been subject to oxidative stress [117]. SP1 is likewise elevated within Huntington disease (HD) [118]. Cell and animal models have shown that mutant huntingtin upregulates the SP1 gene. Blocking this upregulation produced a neuroprotective effect [119]. This indicates that SP1 upregulation may have a general neurodegenerative etiological result, of which AD is one specific variant.

**IMPLICATIONS OF THE LEARn MODEL: PERSONAL HEALTH PRACTICES AND PUBLIC POLICY**

The LEARn model posits environmentally–induced hypomethylation or oxidative damage as the physical mechanism that perturbs gene expression. These perturbations are latent. They are not immediately apparent in the same manner found in conventional toxic responses. Thus, apparent reversal of the symptoms of acute exposure to environmental stressors such as Pb or poor nutrition need not mean that there will be no long–lasting repercussions of an environmental insult. This suggests a possibility for biologically–
based, but non-“heroic” medical remediation. The proposed mechanisms of LEARn, epigenetic hypomethylation and/or oxidative damage to DNA, do lend towards potential solutions to a LEARn-type environmental exposure. For example, fruit juices, such as concentrated apple juice, have been shown to reverse acute oxidative damage and be a useful source of s-adenylmethionine, reversing hypomethylation in C57B1/6J and APOE−/− mice [120]. Dietary supplementation with folic acid has reversed genomic hypomethylation in human patients with colorectal adenoma in a double blind, placebo controlled study [121]. In addition, dietary supplementation with the antioxidant melatonin has been shown to reduce levels of nitric oxide synthase and reactive oxygen species in senescent mice [122]. Likewise, dietary melatonin supplementation reduced levels of Aβ in mouse cerebral cortex [123]. This suggests investigation of the use of appropriate fruit and vegetable dietary supplementation early in life, as a prophylactic or treatment measure against possible latent response to environmental insult [124].

**BROADER APPLICABILITY**

The LEARn model is not meant to be an explanation unique to AD. Several other disorders exist for which elucidation of etiology is still primarily in the range of incomplete risk factors and partially-influential pathways. In addition, linkage between early-life events and neurobiological disorders has previously been demonstrated. Diseases such as schizophrenia have been linked to infection, fetal malnutrition or hypoxia in early life [125-126]. A recent study by Bilbo et al. showed that perinatal exposure to an infectious agent affected how the nervous system responded to a later immune challenge and memory consolidation in adulthood [127]. Furthermore, behavioral studies following developmental exposure to environmental agents such as methyl mercury [128], methylazoxymethanol [129], and triethyltin [130] have provided evidence that delayed latent neurotoxicity is exhibited by animals following chemical exposures. These data suggest that behavioral and neurodegenerative outcomes later in life can be influenced by developmental exposure to environmental agents and that the response to future chemical exposure maybe enhanced by such events.

Autism-spectrum disorders have a long history of “incomplete” genetic effects, with over a dozen supposed “candidate genes”. Work in the field has demonstrated at least some differential methylation contributing to the disorder [131]. Recently our work has indicated the possibility of a sub-type of “autism with aggression” characterized by elevated APP levels [132]. Hypomethylation of the DRD2 and HTR2A genes have been implicated in both schizophrenia and bipolar disorder [133]. Likewise, while the HTTPLR polymorphism has been shown to be connected with neuroticism, bipolar and anxiety disorders, the link has been shown to be inconsistent [134-137]. On the other hand, hypermethylation of the RELN (rellin) gene has been shown to associate with schizophrenia [138], indicating that conditions may still be more complex than a single model can cover. Therefore, the LEARn model is likely to be of broader application than the AD field, alone.

The LEARn model can be seen as a special case of modulation of gene expression by persistent alteration of the structure of a gene without changing its primary DNA sequence. The more general case is the "somatic epitype". The somatic epitype is a non-heritable, non-silent acquisition of non-primary sequence changes in DNA (e.g. changes in methylation patterns) through environmental influences, such as heavy metals, nutrition, behavioral stressors, or intrinsic factors such as changes in oxidative species within a cell [139]. When a somatic epitype is acquired early in life and its influence is latent, the LEARn model would apply. More acute somatic epitypes could also function in the etiology of neuropsychiatric disorders and some cancers.

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**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no competing financial interests.

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