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Review

The Impact of Apolipoprotein E (*APOE*) Epigenetics on Aging and Sporadic Alzheimer's Disease

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Abstract: Sporadic Alzheimer's disease (AD) derives from an interplay among environmental factors and genetic variants, while epigenetic modifications have been expected to affect the onset and progression of its complex etiopathology. Heterozygous carriers of the apolipoprotein E gene (*APOE*)*ε4* allele have a 4-fold increased risk of developing AD, while *APOE ε4*/*ε4*-carriers have a 12-fold increased risk in comparison with the *APOE ε3*-carriers. The main longevity factor is the homozygous *APOE* ε3/ε3 genotype. In the present narrative review article, we summarized and described the role of APOE epigenetics in aging and AD pathophysiology. It is not fully understood how *APOE* variants may increase or decrease AD risk, but this gene is known to affect amyloid- and tau-mediated neurodegeneration directly or indirectly, also by affecting lipid metabolism and inflammation. For sporadic AD, epigenetic regulatory mechanisms may control and influence *APOE* expression in response to external insults. Diet, a major environmental factor, has been significant associated with physical exercise, cognitive function, and the methylation level of several cytosine-phosphate-guanine (CpG) dinucleotides sites of *APOE*.

Keywords: apolipoprotein E; Alzheimer's disease; methylation; dementia; epigenetics; tau protein; amyloid-β; longevity

1. Introduction

The study of complex diseases is based on the association among epigenetics, gene variants, and environmental factors [1,2]. The pathophysiology of Alzheimer's disease (AD) is a mixture of many pathogenic pathways and gene expression networks. The current model of AD is based on the amyloid-β (Aβ) hypothesis, in which a series of deterministic events leads from Aβ and tau deposition to neurodegeneration and progressive decline of cognitive function. This conceptualization matches autosomal-dominant AD, defined as dominantly inherited AD with pathological confirmation, although it is less appropriate for sporadic AD. A probabilistic AD model connoted by three variants of the disease has been proposed: autosomal-dominant AD, apolipoprotein E (apolipoprotein E gene, *APOE*) ε4 allele-related sporadic AD, and *APOE* ε4 allele-

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unrelated sporadic AD [3]. These three variants suggested a reduced weight of the Aβ hypothesis, giving more importance to environmental factors and lower-risk genes [3].

There were epigenetic modifications also in AD [4]. From a genetic point of view, the known risk loci showed a low penetrance in causing AD, except for Aβ production-related genes, and none of them have been related to different AD pathogenic pathways. On the contrary, epigenetic alterations may modify transcriptional activity globally throughout different genes and multiple biological pathways. Epigenetic mechanisms may also explain the influence of environmental stimuli such as dietary patterns, harmful exposures, and lifestyle factors on phenotypic outcomes in individuals with the same genetic variants [5]. Additionally, genetic sequence and epigenetic code were linked in a clear way. In fact, some single nucleotide polymorphisms (SNPs) are considered a common epigenetic mark because of the rearranging of cytosine-phosphate-guanine (CpG) dinucleotides with C nucleotide methylation. These CpG-altering SNPs may modulate DNA methylation levels in a *cis* or *trans* manner or they may modify gene transcription at regions enhanced of CpG known as CpG islands [6–8].

Since early 90's, many studies have showed that *APOE* could play a central role in AD neurodegeneration. For sporadic AD *APOE* allele *ε*4 is a key genetic risk factor [9–11], with a semidominant inheritance [12], and associated to the ApoE4 isoform. Conversely, in sporadic AD, the *APOE* allele *ε*2, associated to the ApoE2 isoform, could have a protective effect [13,14]. Although the increased risk for sporadic AD in *APOE ε*4-carriers, the presence of the *APOE ε*4 allele alone is not a causal factor for AD pathology [15]. In this context, epigenetics may represent a candidate for a point of overlapping among several AD genetic risk factors, such as the *APOE* ε4 allele, and the AD pathophysiological processes. Human ApoE is a glycoprotein of 299-amino acids, traditionally binding phospholipids and cholesterol. ApoE is produced in 3 common isoforms (ApoE2, ApoE3, and ApoE4) differing in two amino acid residues at positions 112 and 158, and one very uncommon isoform (ApoE3r) [16].

The *APOE* variants, respectively ε^2 ε^3, ε^4 and ε^3r, are determined by four haplotypes, derived from the allele association of 2 common SNPs rs429358 ($C^{3,937} \rightarrow T$) and rs7412 ($C^{4,075} \rightarrow T$) at the *APOE* locus (19q13.32), coding for the different protein isoforms [16]. These *APOE* four alleles [17], are considered the most investigated variants in human Caucasian genome. Remarkably, the *APOE* exon 4 region, encompassing the ε2/ε3/ε4 allele variants, is a well-defined CpG islands rich area. Moreover, the two common SNPs rs429358 and rs7412 are CpG-altering and modify the CpG content of this area. This *APOE* CpG islands rich area is a transcriptional enhancer with a specificity linked to the ε4 allele and cell-type [18]. In the present review article, we briefly summarized and highlighted the complex epigenetic regulation of *APOE* gene in aging and sporadic AD.

2. The role of apolipoprotein e in Alzheimer's disease pathogenesis

For sporadic AD, *APOE* is the most important genetic risk factor as well as for the earlier stages of cognitive decline represented by mild cognitive impairment (MCI) [19], but its expression is poorly understood. Astrocytes and activated microglia produced the major amount of ApoE in the brain. Having one *APOE ε4* allele conducts to a 4-fold increased risk of developing AD, while having two *APOE ε4* alleles conducts to a 12-fold increased risk, compared to the *APOE ε3*-carriers. Conversely, the uncommon heterozygous carriers of the *APOE ε2* allele have a risk 40% lower for AD and the homozygous carriers have a further reduced risk [20]. It was showed that *APOE ε4*-carriers with normal cognition displayed elevated Aβ and tau brain burden than *APOE ε3*-carriers; conversely, *APOE ε2*-carriers had reduced global Aβ burden, without differences in regional tau burden or accumulation over time [21]. The contribution in AD pathogenesis from *APOE* involves not only Aβ aggregation and its clearance, but also tau-mediated neurodegeneration [22], microglia impairment [23,24], astrocyte reactivity [25], and blood-brain barrier disruption [26,27].

The three ApoE isoforms bind and transport Aβ peptides with differential affinity during AD pathogenesis [28,29], being highest for ApoE4, intermediate for ApoE3, and lowest for ApoE2 [30,31]. Therefore, their effects are also different concerning Aβ aggregation and clearance, but not Aβ production [32,33]. ApoE also can affect tau-mediated neurodegeneration and tauopathy by

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modulating microglial responses to Aβ plaque pathology [34–36]. Thus, different ApoE isoforms may increase or reduce the risk for AD [29,31], based on different combined effects of ApoE isoforms on both Aβ deposition and neurofibrillary tangles [37]. *APOE* and its ε2/ε3/ε4 alleles have been connected by several genetic studies to multiple physiological conditions and disorders. Epigenetic alterations could explain the association between *APOE* and its associated diseases, considering that genetic signal associated with the disease also reflects a site's sequence architecture for epigenetic code [18].

3. Apolipoprotein E, human longevity, and Alzheimer's disease

There was a genetic association of *APOE* is with both human longevity and AD, but its mechanistic contribution in aging is largely under investigation. *APOE* pleiotropic roles may be explained by its exceptional epigenetic properties. In AD brain, these epigenetic changes could contribute to neural cell dysfunction. Additionally, several studies showed DNA methylation modifications on specific genes implicated in AD pathology such as *APOE*. In AD brain, it was showed that *APOE* CpG islands were differentially methylated in an *APOE*- and tissue-specific way [38]. In the brain of targeted replacement (TR) mice expressing human ApoE, allele variations within the major *APOE* CpG island may affect its methylation [39]. Epigenetic changes may link modified gene expression with environmental stimuli such as dietary patterns and physical exercise. In animal models, *APOE* alleles may have alterations in epigenetic regulation in response to external stimuli reported in studies on *APOE* TR mice [40].

The differences between mouse and human *APOE* gene clusters, the complexity of transcriptional control of human ApoE, and the structure of the targeting construct should be considered in the strategy for replacing mouse ApoE in the *APOE* TR models [41]. Moreover, lifestyle factors like education, alcohol consumption, smoking, and physical activity may attenuate genetic risk in the process of age-related cognitive decline and twelve modifiable risk factors might prevent or delay up to 40% of different dementias [42]. The complex interactions among age-related cognitive decline, genetics, and lifestyle may encourage behaviors maintaining cognitive health in older age [43]. At this regard, ApoE may be important for the pathophysiology of lipid metabolism [44] and central nervous system (CNS), although the role in healthy aging and longevity has seen its value growth [45–47].

Studies on longevity and healthy aging are related because subjects who live long tend to be healthy for a greater part of their lives [48]. Healthy aging can be defined as achieving older age maintaining intact cognition and/or mobility and without disabilities or multimorbidity. This last can be defined as the coexistence of two or more chronic diseases in the same subjects [49]. The detrimental effects of the *APOE* ε4 allele on longevity could influence the probability of a long human lifespan [48]. The *APOE* ε2 allele is more frequent in long-lived individuals than the ε4 allele [50]. Thus, the main longevity factor is the homozygous *APOE* ε3/ε3 genotype. The higher frequency of the ε3 allele in older individuals and their offspring than in controls derives from the greater amount of *APOE* ε3/ε3 genotype compared to the ε2/ε3 or ε3/ε4 genotypes [51].

In the pathophysiology of lipid metabolism, the role of ApoE may be related with normal/pathological aging, while its function in the pathophysiology of CNS needs further clarification [52]. In fact, in the CNS, there was about a quarter of total body cholesterol that may exert an important role in synaptic plasticity [53]. With advancing age, cholesterol metabolism may modify, and its related brain changes may be associated with the pathophysiology of AD [53]. So, in longevity and healthy aging, lipid and cholesterol maintenance are a critical factor also from an interventional point of view. The detrimental effects of *APOE ε*4 allele might be managed by dietary interventions [54], with a Mediterranean dietary pattern potentially including higher n-3 polyunsaturated fatty acid intakes [55,56].

4. Specific epigenetic modifications of apolipoprotein E in Alzheimer's disease

In response to environmental stimuli, epigenetic marks and signals may enable temporal combination of regulatory events through mechanisms including DNA methylation, histone

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modification/chromatin conformation, and noncoding microRNAs (miRNAs). Several studies investigating DNA methylation in the *APOE* gene suggested an age-dependent flow and *APOE* DNA methylation specific for brain area. The *APOE* genomic sequence is approximately 4 kb in size (chromosome19:45408714-45412650, hg19) including its promoter. This region encompasses 172 CpG dinucleotides [57]. In the late 90s and early 2000s, polymorphic sites in the first intron and the proximal promoter the of *APOE* gene cluster (−1,019 to +407) affecting *APOE* expression have been identified [58–64] (Table 1). Notably, these polymorphisms have been related with a differential AD risk [65,66]. However, in AD, the association between these polymorphic sites and the variability of sequence in the proximal promoter with ApoE protein levels were not clearly understood. In fact, among different studies, findings on the levels of expression of *APOE* RNA and the relationship with the ApoE levels varied. In human *postmortem* brain, there was elevated methylation in AD frontal lobe of a 5′-C-phosphate-G-3′ (CpG) island overlapping with exon four and downstream [67]. Interestingly, *APOE* has a well-defined CpG island not residing in the promoter region and overlapping with the *APOE* 3′-exon. In the human genome, these 3′-CpG islands are very rare representing < 1% of total CpG islands and are also conserved in other mammals [68,69]. However, the *APOE* CpG island methylation level relates to the expression level of four known *APOE* transcripts. The majority of the total *APOE* mRNA, with higher expression in the AD frontal lobe than in the frontal lobe of control subjects, is constituted by circular RNAs, miRNAs, and truncated *APOE* transcripts. The findings of several studies suggested several changes in epigenome and the regulatory role of epigenomic elements associated with the risk or clinical presentation of different neurological diseases, although the exact clinical significance of these signatures in the quantities of RNA and methylation level of CGI in the *APOE* 3′-exon was still unclear [67] (Table 1).

At the level of the individual CpG site, epigenetic regulation was showed by up/down patterns in the methylation profiles between samples and tissues. Significant differences in the global methylation levels among several brain regions were discovered across *postmortem* brain tissues. In brain regions primary affected by AD such as frontal lobe, temporal lobe, and hippocampus, methylation levels were lower. Conversely, in the cerebellum, a region apparently lacking profound pathological changes in AD but with recent important findings, the highest methylation levels were observed, suggesting that a correlation may exist between the methylation levels of the *APOE* CpG islands and the vulnerability of brain AD regions [70]. In fact, age- and AD-related alterations in several cerebellar subregions may also impact numerous functional domains, especially those affecting cognitive processing [70].

Genetic variants, which consist of CpG-altering SNP, can modify DNA methylation levels. These genetic variations may act like regulatory elements connecting genetic changes with epigenetic variability [71] (Table 1). As previously described, the *APOE* ε2/ε3/ε4 alleles are produced by two CpG-altering SNPs (rs429358 and rs7412) residing within the core region of the *APOE* CpG islands. The *APOE* ε4 allele, if compared with ε2 or ε3 alleles, adds one more CpG, further saturating a small 12 bp region with 4 CpG sites. On the contrary, the *APOE* ε2 allele eliminates 1 CpG and opens a 33-bp CpG-free region. Therefore, these two SNPs may alter the regional CpG burden and probably influence global DNA methylation of the CpG islands. These CpG load changes might change the binding profiles of methyl CpG-binding domain proteins, connected specifically to methylated DNA through their exclusive amino acid pattern [72].

Furthermore, within the *APOE* CpG islands, there is evidence of indirect indicators of protein binding which consist of histone marks and a DNase I hypersensitivity cluster. These findings suggested that the *APOE* CpG islands (and exon 4) may be a site for chromatin remodeling and protein binding. Considering that environmental stimuli could influence DNA methylation gradually with aging, the differences in *APOE* CpG islands methylation between healthy individuals and AD increased with age [73]. Taken together, different methylation landscapes could be represented by inheritance of different ε2/ε3/ε4 alleles in the *APOE* CpG islands, which could accumulate or change continuously with age, also modified by environmental factors. Recent results showed that methylation levels for most CpG sites may be in the order of *APOE* ε4-carriers > *APOE* ε3/ε3-carriers > *APOE* ε2-carriers, considering that *APOE* e4-carriers have the greatest number of

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CpG sites, while *APOE* e2 carriers have the smallest number with ε3/ε3 in the middle [74] (Table 1). These changes could potentially alter protein binding, with some consequences on biological systems, even affecting the pathophysiological processes of multiple diseases and plasma lipids levels. *APOE* methylation could partially mediate the effects of age on plasma lipid (Figure 1).

Table 1. Overview of studies illustrating epigenetic signatures of apolipoprotein E gene (*APOE*) in aging and Alzheimer's disease (AD).

***APOE* exons, promoter, and CGI**				
Study	**Study design**	**Sample size**	**Age or mean age at death (years)**	**Principal findings**
Lambert et al., 1998 [59]	Cross-sectional	AD: 573 Controls: 509	AD: 73.8±8.1 Controls: 70.4±7.9	Among three *APOE* promoter mutations (−491 AT, −427 CT and Th1/E47cs), the Th1/E47cs T allele was associated with an increased AD risk, while the −491 T allele was associated with a decreased risk, independently of the *APOE* ε2/ε3/ε4 polymorphism effect. The −427 CT polymorphism was not associated with AD. In addition to the qualitative effect of the *APOE* ε2/ε3/ε4 polymorphisms on the AD occurrence, the quantitative variation of expression of these alleles due to functional *APOE* promoter mutations, may be a key determinant of AD development
Lambert et al., 1998 [61]	Cross-sectional	AD: 310 Controls: 293	AD: 72-91 Controls: 75-102	The Th1/E47cs T allele was associated with an increased risk of developing AD (odds ratio, OR = 1.29) and the OR was 1.79 for individuals bearing at least one T allele
Yu et al., 2013 [71]	Cross-sectional	Frontal lobe AD: 9 Controls: 6	Frontal lobe AD: 86.8±6.9 Controls: 87.9±8.6	*APOE* CGI exhibited transcriptional enhancer/silencer activity and differentially modulates expression of genes at the *APOE* locus in a cell type-, DNA methylation- and ε2/ε3/ε4 allele-specific manner. These findings implicated a novel functional role for a 3′-exon CGI and supported a modified mechanism of action for *APOE* in disease risk, involving also an epigenetically regulated transcriptional program at the APOE locus driven by the *APOE* CGI
Lee et al., 2020 [67]	Cross-sectional	Frontal lobe AD: 44 Controls: 21 Cerebellum AD: 51 Controls: 25	Frontal lobe AD: 86.8±6.9 Controls: 87.9±8.6 Cerebellum AD: 74.6±9.3 Controls: 73.5±10.9	*APOE* has a single CpG island (CGI) that overlaps with its 3′-exon. In this study, the presence of *APOE* circular RNA (circRNA) was discovered and found that circRNA and full-length mRNA each constitute approximately one third of the total *APOE* RNA, with truncated mRNAs likely constituting some of the missing fraction. All *APOE* RNA species demonstrated significantly higher expression in AD frontal lobe than in control frontal lobe, suggesting a possible modified mechanism of gene

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				action for *APOE* in AD involving also an epigenetically regulated transcriptional program driven by DNA methylation in the *APOE* CGI
Ma et al., 2015 [74]	Cross-sectional	475 men and 518 women	18-87	The 13 APOE CpG sites were categorized into three groups: Group 1 showed hypermethylation (> 50%, in the promoter region), Group 2 exhibited hypomethylation (< 50%, in the first two exons and introns), and Group 3 showed hypermethylation (> 50%, in the exon 4. *APOE* methylation was significantly associated with age and plasma total cholesterol and *APOE* methylation patterns differed across *APOE* ε variants and the promoter variant rs405509, which further showed a significant interaction with age

CGI: 5′-C-phosphate-G-3′ (CpG) island; CpG: cytosine-phosphate-guanine.

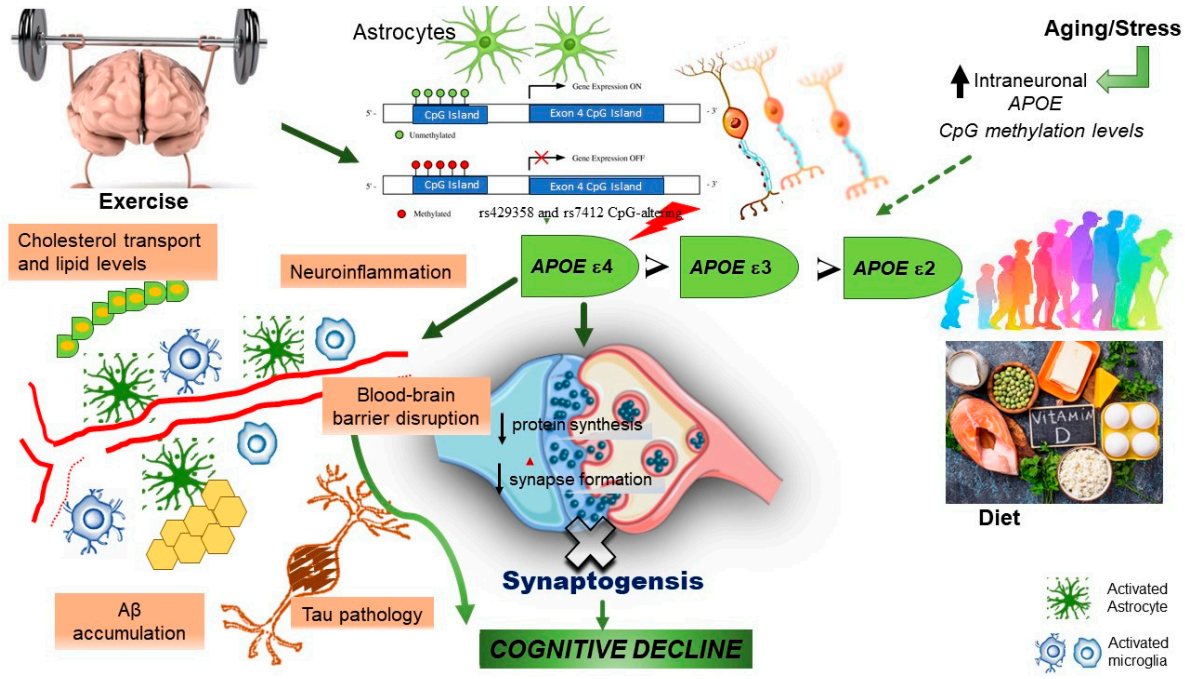

Figure 1. Despite the high lifetime risk linked to the presence of the apolipoprotein E (*APOE*) ε3/ε4 and *APOE* ε4/ε4 genotypes (the greatest risk factor for developing Alzheimer's Disease, AD), stochastic factors (such as environment, diet, physical exercise, and ageing), may play a significant role. *APOE* pleiotropic roles may be explained by its exceptional epigenetic properties. The APOE ε2/ε3/ε4 alleles are produced by two cytosine-phosphate-guanine (CpG)-altering SNPs (rs429358 and rs7412) residing within the core region of the APOE CpG islands. *APOE* ε4 carriers have the greatest number of CpG dinucleotides sites, while *APOE* ε2 carriers have the smallest number, so methylation levels for most CpG sites are in the order of *APOE* ε4 carriers > *APOE* ε3/ε3 > *APOE* ε2 carriers. The role of *APOE* in AD pathogenesis involves not only amyloid-β (ab) aggregation and clearance, but also tau-mediated neurodegeneration, microglia dysfunction, astrocyte reactivity, and blood-brain barrier disruption.

In the epigenetic landscape, miRNAs are known to be small non-coding RNAs with a length of ~ 22 nucleotides They are also implicated in AD, as showed by the altered expression of miRNA 650

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(miR-650) in AD brains [75]. Bioinformatic analysis showed that miR-650 may target the expression of three components associated to AD: *APOE*, presenilin 1 (PSEN1), and cyclin-dependent kinase 5 (CDK5), with recent findings confirming that miR-650 may reduce *in vitro* the expression of *APOE*, PSEN1, and CDK5 [75].

5. Epigenetics of apolipoprotein E and cognitive function: contrasting evidence in Alzheimer's disease

Several lifestyle and environmental stimuli could explain the effects of *APOE* genotype on AD and cognitive functioning, such as exercise [76], education [77], and vitamin D status [78]. Among implications for the development and progression of AD, vitamin D supplementation may be another potential strategy to consider for the *APOE* ε4 allele-carriers. Some reports showed that higher vitamin D concentrations in *APOE* ε4 homozygous carriers allow to perform better at memory scores [79]. Then, compared to the *APOE* ε3/ε3-carriers, the *APOE* ε4-carriers showed earlier onset of cognitive impairment in AD. Although, after the disease onset, the effect of *APOE* genotype on the progression of cognitive impairment remained debated [80].

For this reason, epigenetic modifications of *APOE* such as DNA methylation have a central role in maintaining cognitive function in older age. Growing DNA methylation levels at the *APOE* promoter region were found on *postmortem* prefrontal cortex samples of sporadic AD individuals by mass spectrometry [81]. Numerous previous studies have investigated the association between *APOE* DNA methylation and AD or MCI [82–84]. Instead, the association between *APOE* DNA methylation and cognitive function in healthy subjects without cognitive impairment was evaluated by two studies with controversial findings [85,86]. Liu and colleagues found an inverse association between DNA methylation in the *APOE* gene region and delayed recall capacity among 289 older African Americans people with a mean age of 67 years during normal cognitive aging [85]. Conversely, the other study conducted in a large European cohort, observed no association between general cognitive functioning and *APOE* DNA methylation [86].

Many reports have suggested that neuroinflammation may have a key role in AD pathogenesis [87]. Dietary habits are known to influence systemic inflammation, neuroinflammation, and inflammaging [88]. A recent study conducted in a cohort of racially diverse middle-aged people (n = 411), pursued to identify DNA methylation sites associated with cognitive function in the genomic region of *APOE*. About inflammatory potential of the diet, among the dietary inflammatory index, cognitive performance, and the methylation level of several CpG sites have been detected significant relationships [89].

However, studies are contrasting at this regard, and if epigenetic biomarkers could be used for predicting AD is still unclear. In the *APOE* gene, DNA methylation at two CpG sites (3/13) that are known to show age-dependent changes, was related with the total cholesterol and high-density lipoprotein cholesterol ratio, but not with cognitive status, family history of AD, or the risk of cardiovascular disease in a blood-based DNA methylation study of 5828 people from the Generation Scotland cohort [90]. These findings supported that there is no evidence yet for considering *APOE* methylation as a biomarker for predicting AD or cardiovascular disease, although *APOE* methylation was associated with the blood levels of cholesterol [90].

6. Conclusions

In the panorama of current available evidence, the investigation of healthy aging and longevity is currently of remarkable interest. *APOE* could be considered an epigenetic mediator of senescence considering that different ApoE biochemical pathways in lipid metabolism, neuroinflammation and neurodegeneration may contribute to longevity and healthy aging. Nonetheless, such areas of investigation are still increasing, since ApoE function in neurodegenerative diseases such as AD cannot be uniquely explained by ApoE effects in lipid metabolism. Furthermore, the imbalance in the ApoE isoforms could explain the pathophysiological process of cognitive impairment linked to sporadic AD [91].

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Stochastic factors (such environmental, diet, and pollution) may play a significant role in sporadic AD, despite the elevated lifetime risk linked to *APOE* ε3/ε4 and *APOE* ε4/ε4 genotypes. Indeed, according to the notion of stochastic risk or protective factors and although it is known that *APOE* ε4/ε4-carriers developed dementia about 10 years earlier than *APOE* ε2 carriers [92], there was still significant discrepancy in the age of onset for *APOE* ε4/ε4-carriers (standard deviation of 6 years) [93]. During the process of aging, the accumulation of molecular alterations driven by genetic and epigenetic events in the organism lead to a loss of phenotypic plasticity over time. Also, epigenetics may be altered during the process of aging, and it is particularly important as age is the greatest risk factor for developing AD [94].

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: For this type of study, formal consent is not required.

Conflicts of Interest: The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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