

REVIEW

# Apolipoprotein E4: A Risk Factor for Successful Cognitive Aging

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Apolipoprotein E is a plasma protein that has an important role in transport and metabolism of lipids in serum as well as central nervous system. Among the 3 common alleles, the  $\epsilon 2$  allele has the most stable structure followed by  $\epsilon 3$  and  $\epsilon 4$  in order. There is evidence for a deleterious role of  $\epsilon 4$  allele by atherosclerosis and amyloid beta accumulation in brain and body. The presence and gene dose of  $\epsilon 4$  allele are risk factors for late-onset Alzheimer's disease have a role in the pathology of amyloid beta and tau and it has a strong relationship with the early onset of late-onset Alzheimer's disease. However, early-onset Alzheimer's disease has a weaker relationship with  $\epsilon 4$  allele of apolipoprotein E.

**Key Words** apolipoprotein E, polymorphism, Alzheimer's disease.

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## INTRODUCTION

The human apolipoprotein E (apoE, protein; APOE, gene) is single chain protein, classified as a lipoprotein, with 299 amino acids. ApoE has 2 domains i.e., the amino-terminal domain that has low-density lipoprotein receptor binding region and a carboxy-terminal domain that has lipid-binding region.<sup>1</sup> APOE gene, located on chromosome 19q13, has several single-nucleotide polymorphisms.<sup>2</sup> The 3 common type polymorphisms are  $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ; whereas rare type polymorphisms include  $\epsilon 1$ ,  $\epsilon 5$ ,  $\epsilon 7$ . The common types constitute 3 homozygous ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 3/\epsilon 3$ ,  $\epsilon 4/\epsilon 4$ ) and 3 heterozygous ( $\epsilon 2/\epsilon 3$ ,  $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 4$ ) diplotypes, which induces either exchange of 1 or 2 amino acid(s)<sup>3,4</sup> or glycosylation of 1 amino acid.<sup>5</sup> The  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  alleles differ by single amino acid substitutions at residues 112 and 158 of the protein. The amino acids sequence of these residues is cysteine-cysteine ( $\epsilon 2$ ), cysteine-arginine ( $\epsilon 3$ ), and argi-

nine-arginine ( $\epsilon 4$ ).<sup>6</sup> The  $\epsilon 3$  allele also is the most common allele in all population and neutral or protective to cells and organs.

ApoE is an important plasma protein found in plasma lipids such as very low-density lipoproteins, chylomicron, and a subclass of high-density lipoprotein. It is essential for the catabolism of triglyceride-rich lipoprotein constituents, transportation of cholesterol and other lipids, and cellular repair.<sup>7,8</sup> Foods with high cholesterol and triglyceride induce its expression in various animals.<sup>9</sup> The liver is the main organ producing apoE in human, producing >75% of total apoE. Brain, spleen, lung, kidney, ovary, testis, peripheral nerves and muscle also produce apoE.<sup>10</sup> Cholesterol is associated with myelin production and essential component of the brain cell membrane. It contributes to brain development, neuronal maintenance, and repair, as well as maintaining the synaptic plasticity of neuron cells.<sup>11</sup> Astrocytes and microglia, vascular smooth muscle cells, and choroid plexus are sources of apoE in the human brain. Neurons can produce apoE, especially under stressful conditions.<sup>7,12</sup> Increased apoE can modulate lipid metabolism in the compromised nervous system.

APOE polymorphism was identified in 1993 in relationship

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to the onset and clinical feature of Alzheimer's disease. It has since become an important factor in the understanding of pathophysiology of Alzheimer's disease, immunoregulation, and cognition in other dementias.<sup>13</sup>

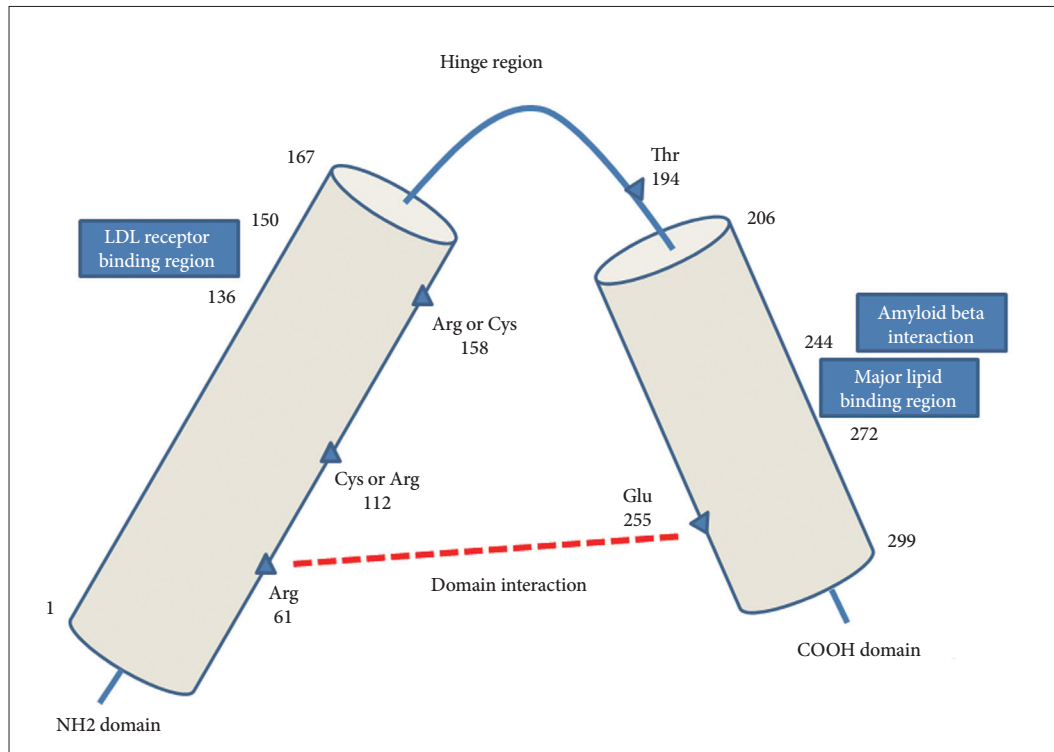
Whether APOE has a protective or harmful role in the brain is under debate.<sup>14,15</sup> In an epidemiological study, the terms are relative.<sup>16-18</sup> When the APOE  $\epsilon 4$  allele frequency is higher in patients with Alzheimer's disease than cognitively normal person, APOE  $\epsilon 4$  is considered harmful. On the other hand, when the frequency of APOE  $\epsilon 2$  allele in Alzheimer's disease patients is less than that of cognitively normal person, APOE  $\epsilon 2$  allele may be protective.<sup>19</sup> However, these terms have a different meaning in laboratory studies. When cells with APOE  $\epsilon 2$  allele survive longer than cells with other haplotypes in a toxic environment, then APOE  $\epsilon 2$  is protective. APOE  $\epsilon 4$  is considered toxic to the nervous system and vascular endothelial cells, as compared to the other isoforms. The biological efficacy of APOE  $\epsilon 3$  is between APOE  $\epsilon 2$  and APOE  $\epsilon 4$ , hence, APOE  $\epsilon 3$  is considered neutral in terms of risk for Alzheimer's disease.<sup>14,20</sup>

The mechanisms for the harmful effect of APOE  $\epsilon 4$  are as follows. First, "domain interaction" theory explains the negative role of the APOE  $\epsilon 4$ .<sup>21</sup> The domain interaction occurs be-

tween Arg-61 of the amino domain and Glu-255 of the carboxy-domain. This single amino acid interchange of the APOE  $\epsilon 4$  causes a structural change such that APOE  $\epsilon 4$  becomes more compact than APOE  $\epsilon 3$  or APOE  $\epsilon 2$ .<sup>22</sup> This mediates the adverse effects of APOE  $\epsilon 4$  (Fig. 1).<sup>23</sup> Second, affinity of APOE  $\epsilon 4$  for very low-density lipoproteins and low-density lipoprotein could explain brain damage by APOE  $\epsilon 4$ .<sup>24</sup> Third, a recent study confirmed that proteolytically cleaved APOE  $\epsilon 4$  is a major factor in Alzheimer's disease. An amino-terminal fragment of APOE  $\epsilon 4$  is identified in neurofibrillary tangles using antibody, suggestive of neurotoxic effect of the amino terminal.<sup>25,26</sup> Finally, the carboxy-domain fragments of APOE  $\epsilon 4$  are neurotoxic and cause mitochondrial dysfunction and formation of neurofibrillary tangles in transgenic mice.<sup>27</sup>

## ALZHEIMER'S DISEASE

Alzheimer's disease is the most common cause of dementia in the elderly.<sup>28</sup> With an increment of life expectancy in developed countries, the incidence and prevalence of Alzheimer's disease are significantly rising. The prevalence of Alzheimer's disease is increasing roughly at 2-fold rate per 5 years in patients above 65 years of age, reaching >30% at age 85. Al-



**Fig. 1.** Schematic diagram of human apolipoprotein E structure and main functional areas. The NH2 terminal domain and COOH terminal domain is connected by a flexible hinge region. There is low-density lipoprotein receptor binding region in NH2 terminal domain while Major lipid binding region and Amyloid beta interaction region are in COOH terminal domain. In apolipoprotein E  $\epsilon 4$ , domain reaction occurs between Arginine at residue 61 and Glutamate at residue 255 which results in the compact shape of the molecule. LDL: Low-density lipoprotein.

Alzheimer's disease is considered to have heterogeneous genetic causes. It is divided into early-onset Alzheimer's disease and late-onset Alzheimer's disease by the age 65. Interestingly, Alzheimer's disease with the strong genetic background, usually autosomal dominant, has a relatively early onset of around 50 years. Moreover, research with genome-wide association study reveals that APOE  $\epsilon 4$  carriers have a 33-fold higher risk of Alzheimer's disease than APOE  $\epsilon 3/3$  carriers.<sup>29,30</sup> Loss of short term memory is the earliest clinical feature followed by loss of other cognitive features such as visuospatial function, language function, and frontal executive function. The primary pathology of Alzheimer's disease is an abnormal aggregation of amyloid beta that is produced from amyloid precursor protein<sup>31</sup> in the extracellular space and tau protein in the neuronal cell.<sup>32</sup> Accumulation of amyloid beta causes senile plaque and accumulation of abnormal tau protein causes neurofibrillary tangle. The major component of neurofibrillary tangles is hyperphosphorylated tau, a form of paired helical filament.<sup>33-35</sup> APOE  $\epsilon 4$  is precisely correlated with cerebrospinal fluid amyloid beta levels in the preclinical stage of Alzheimer's disease, which is less prominent in full-blown dementia.<sup>36</sup> APOE  $\epsilon 4$  may also mediate the development of dementia through tau phosphorylation, destruction of cytoskeleton, and mitochondrial dysfunction.<sup>37-39</sup> Experiments with cellular models, animal models, and patient biomarkers suggest that amyloid beta induces tau pathology. However, the relationship between amyloid beta and tau protein and their respective role(s) in Alzheimer's disease remains unclear.<sup>40,41</sup> Perivascular accumulation of amyloid beta also leads to other pathologies such as cerebral amyloid angiopathy.<sup>42</sup> Longitudinal neuroimaging and pathological studies show that pathological changes of Alzheimer's disease begin decades before the clinical onset.<sup>43-46</sup> Excess aggregation of amyloid beta is a major shift in early stage Alzheimer's disease. Amyloid beta 40 and 42 are important components among its subtypes. Amyloid beta 40 is more prevalent and less toxic than Amyloid beta 42.<sup>47</sup> Amyloid beta associated senile plaques and hyperphosphorylated tau associated neurofibrillary tangles are possibly associated with APOE  $\epsilon 4$ .<sup>48-51</sup> These pathological changes result in loss of dendritic spines and decrement of synaptic density, finally, neuronal cells' death.<sup>52,53</sup> While the former study supports a harmful role of APOE  $\epsilon 4$ ,<sup>37-39</sup> later study suggests that multiple factors modulate the effect of APOE  $\epsilon 4$  in the development of Alzheimer's disease.<sup>50,51</sup>

Three recent studies explained the discrepancy between the amount of amyloid beta and cognitive dysfunction. A study of gene expression in the cerebral cortex of APOE  $\epsilon 4$  carriers and late-onset Alzheimer's disease indicates several regulatory mediators including APBA2, FYN, RNF219, and SV2A of which,

those involved in amyloid beta precursor protein metabolism are likely to be associated with pathologic changes in late-onset Alzheimer's disease.<sup>54</sup> The longitudinal study, Alzheimer Disease Neuroimaging Initiative (<http://www.adni-info.org>) likewise shows that APOE  $\epsilon 4$  participates in the pathology of pre-clinical Alzheimer's disease via amyloid beta. They also found a significant relationship between cerebrospinal fluid amyloid beta and cerebrospinal fluid clusterin as well as cerebrospinal fluid amyloid beta and cerebrospinal fluid phosphorylated tau on entorhinal cortex atrophy rate. Thus, phosphorylated tau protein and clusterin, a chaperone glycoprotein, mediate neurodegeneration.<sup>55</sup>

Thirdly, hippocampal oscillation of theta and gamma rhythms are possibly associated with cognition. Animal models indicate that hippocampal and cortical network undergo reorganization in Alzheimer's disease. Altered oscillation of theta and gamma rhythm develops first followed by increased amyloid burden, and finally loss of gamma-amino-butyric-acidergic neurons. Moreover, high levels of amyloid beta in hippocampus cause seizure activity without serious neuronal loss.<sup>56,57</sup>

However, lowering tau reduce the cognitive deficit under elevated amyloid beta level by blocking ectopic cell cycle re-entry.<sup>58,59</sup>

## LATE-ONSET ALZHEIMER'S DISEASE

Late-onset Alzheimer's disease is multifactorial, including genetic and environmental factors with negative impact on endocytic function, lipoprotein signaling as well as synaptic regulation.<sup>60</sup> Recently, chronic inflammation causing focal accumulation of mitochondria suggested as a triggering factor for late-onset Alzheimer's disease.<sup>61</sup> Usually, late-onset Alzheimer's disease develops after the age of 65 years, and 60 years is proposed as the more appropriate cut-off age of the illness.<sup>28,62</sup> Amyloid plaque and neurofibrillary tangle are the main pathological findings of late-onset Alzheimer's disease. APOE  $\epsilon 4$  occurs in up to 80% of late-onset Alzheimer's disease patients and is considered a risk factor for this dementing illness.<sup>13,16,62</sup> The following study shows that apoE strongly binds amyloid beta and APOE  $\epsilon 4$  is the common haplotype in late-onset Alzheimer's disease.<sup>49</sup> Moreover, carriers of APOE  $\epsilon 4$  showed up to 15 years earlier disease onset and increased incidence of neuropsychiatric symptoms.<sup>63,64</sup> Intriguingly, two African populations with high frequencies of APOE  $\epsilon 4$  show no such strong relationship.<sup>30,65</sup> In late-onset Alzheimer's disease, the function of key amyloid beta processing enzymes is normal.<sup>60</sup> However, the amount of amyloid plaque in the brain increases not because of increased amyloid beta production but

because of impaired clearance.<sup>66</sup> APOE  $\epsilon$ 4 is responsible for reduced amyloid clearance in the diseased brain.<sup>67</sup> The presence of APOE  $\epsilon$ 4 also related with more rapid progression and poor response to cholinergic therapy in many ethnic groups.<sup>13</sup> However, results are unequivocal in only Caucasian populations; adequate evidence in other ethnic groups such as African American and Hispanic populations is still needed. Relatively smaller sample size, allele frequency variation among ethnicities, and lifestyle issues could explain the discrepancy.<sup>18,68-70</sup> Overall, APOE polymorphism is not a useful diagnostic biomarker or prognostic factor for late-onset Alzheimer's disease, as compared to amyloid beta 42 and tau in cerebrospinal fluid.<sup>71</sup> However, it still may be used as a predictor of increased neuropsychiatric symptoms and decreased response to pharmacological therapy.<sup>13,15,64</sup>

## EARLY-ONSET ALZHEIMER'S DISEASE

Early-onset Alzheimer's disease develops before 65 years old, and it is rare disease composing <1% of Alzheimer's disease cases.<sup>62</sup> Alzheimer's disease was first reported by Dr. Alois Alzheimer in the early 20th century as a case of early-onset disease.<sup>72</sup> Since Corder et al.<sup>13</sup> reported APOE  $\epsilon$ 4 as a risk factor for late-onset Alzheimer's disease, much effort is made to clarify the relationship between early-onset Alzheimer's disease and APOE  $\epsilon$ 4. However, the results are inconclusive. Instead, other genes affecting amyloid precursor protein processing are highlighted and evaluated for a possible relation with early-onset Alzheimer's disease.<sup>62</sup> These include amyloid precursor protein, presenilin-1, and presenilin-2, which are associated with early-onset autosomal dominant Alzheimer's disease. Mutations in amyloid precursor protein gene are related to the conversion of amyloid precursor protein to a better substrate of beta-secretase. Amyloid beta derived from mutant amyloid precursor protein is more easily aggregated than that from wild type. Patient with presenilin mutation usually develops Alzheimer's disease between 30 and 50 years of age. Presenilin mutations were initially thought to increase gamma-secretase activity. However, recent studies reveal that these mutations decrease gamma-secretase activity but increase the ratio of amyloid-beta42/amyloid-beta40, which supports the loss of function hypothesis.<sup>73</sup>

In contrast to the 2 enzymes, alpha-secretase reduces amyloid beta production in the brain. Increased brain APOE  $\epsilon$ 4 has an association with enhanced beta--secretase activity and subsequently increased amyloid beta production.<sup>54</sup> Endosome dysfunction is now considered to have a major role in the production of large amount of amyloid beta in sporadic Alzheimer's

er's disease. A postmortem study shows that enlarged endosomes facilitate a higher chance of amyloid cleavage by beta and gamma-secretase before the development of clinical dementia in APOE carriers.<sup>74</sup> However, endosomal abnormalities are absent in early-onset familial Alzheimer's disease.<sup>75</sup> This result suggests differential mechanisms between early-onset and late-onset Alzheimer's disease. Overall, APOE polymorphism appears to have a limited role in early-onset Alzheimer's disease.

## APOE POLYMORPHISM IN DIFFERENT ETHNIC GROUPS

APOE polymorphism in various ethnic groups is based on the specific disease-status of the group. APOE status follows Mendelian inheritance, with regional as well as ethnic difference. In a haplotype analysis study, APOE  $\epsilon$ 4 was suggested as the ancestral allele in humans.<sup>76</sup> According to this theory, APOE  $\epsilon$ 3 and APOE  $\epsilon$ 2 evolved from APOE  $\epsilon$ 4, but APOE  $\epsilon$ 4 remained after this evolution. Interestingly, apoE amino acid sequence of chimpanzee, genetically closest to humans, is monomorphic, similar to the human APOE  $\epsilon$ 3.<sup>77</sup> Reduced frequency of APOE  $\epsilon$ 4 is a major factor for increased human lifespan with a risk reduction of Alzheimer's disease and cardiovascular disease.<sup>30</sup> As the amount of dietary fat and cholesterol increased during the ancient history of mankind, APOE  $\epsilon$ 3, which can reduce increased cholesterol level with APOE  $\epsilon$ 4, may evolve.<sup>78</sup> Allele frequency of APOE  $\epsilon$ 4 in human population is uneven, with high frequencies of APOE  $\epsilon$ 4 in the equatorial area and high latitudes areas.<sup>79</sup>

In a Korean study of patients with Alzheimer's disease, the most common APOE allele is APOE  $\epsilon$ 3 (71.3%), followed by APOE  $\epsilon$ 4 (21.3%), APOE  $\epsilon$ 2 (7.5%).<sup>18</sup> A population study with normal elderly Korean showed that the most common APOE allele is APOE  $\epsilon$ 3 (86.9%), followed by APOE  $\epsilon$ 4 (6.6%), APOE  $\epsilon$ 2 (6.5%).<sup>80</sup>

In general, Caucasians and Africans have higher frequencies of APOE  $\epsilon$ 4 than Asians. APOE  $\epsilon$ 3 is most commonly found in the majority of populations with a range of 8.5 to 98 percent, followed by  $\epsilon$ 4 (0 to 50%), and  $\epsilon$ 2 (0 to 37.5%).<sup>18,79</sup> These variations of APOE polymorphism among areas in the world and ethnicities could affect the results of clinical studies and drug efficacies.

## CONCLUSION

APOE interacts with environmental and genetic factors in the onset and progression of neurodegenerative diseases as well as cognitive decline. Genotyping APOE polymorphism is



a traditional method for evaluation of dementia and neurodegenerative diseases. Evidence strongly suggests APOE  $\epsilon 4$  as a risk factor for late-onset Alzheimer's disease, but the low predictive value prevents it from usage in diagnosis and prognosis. There is less evidence of APOE genotype as a risk factor for early-onset Alzheimer's disease. Although APOE genotyping itself does not precisely predict the development of a particular disease, it can be included in the fully integrated evaluation of neurological diseases. Preventing the toxic effect of APOE  $\epsilon 4$  may be a method of prevention and treatment of dementing illnesses.

### Conflicts of Interest

The authors have no financial conflicts of interest.

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