Central Asian Journal of Medical and Pharmaceutical Sciences Innovation **1**(5): 205-217 (2021)

NARRATIVE REVIEW

Alzheimer's disease as a neurodegenerative disorder and the apolipoprotein E gene as its genetic biomarker

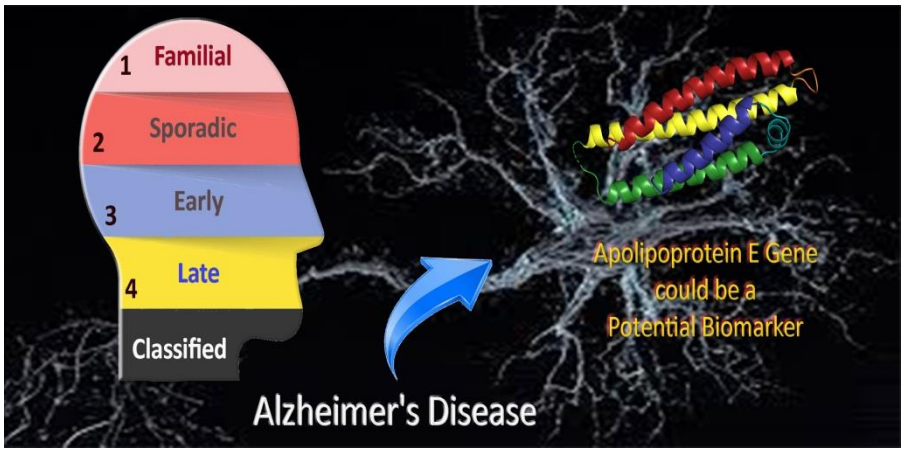
Amirhossein Sahebkar [1,2], **Farnaz Mohajertehran** [3,4,*], **Aida Goloobi** [5,6]

[1] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Oral and Maxillofacial Pathology, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Oral and Maxillofacial Disease Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[5] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[6] Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Highlights

- Alzheimer's disease is a progressive brain disorder that can impair memory and thinking and functional skills.
- Alzheimer's disease is divided into two types, familial and sporadic, and on the other hand, it is divided into two types, early and late.
- Inflammation and oxidative stress are the two main mechanisms involved in Alzheimer's disease.
- Mutations and variants of the apolipoprotein E gene could be a potential biomarker for Alzheimer's disease.

Graphical Abstract



Article Info

Receive Date: 19 July 2021
Revise Date: 31 August 2021
Accept Date: 04 September 2021
Available online: 25 September 2021

Keywords:
Alzheimer disease
Inflammation
Oxidative stress
Apolipoprotein E
Biomarker

Abstract

Alzheimer's disease (AD) is a progressive brain disorder that slowly destroys memory and thinking and functional skills. Alzheimer's disease is classified into two familial and sporadic types. Alzheimer's disease is also divided into the early or late onset of symptoms, with early-onset before age 65 and late-onset after this age. Symptoms of this disease appear in 4 stages: pre-dementia, early, middle and advanced. Many factors are involved in the pathophysiology of this disease. These include oxidative stress, inflammation, and genetics. Oxidative stress occurs with the involvement of beta-amyloid in mitochondrial function. Inflammation also results from the release of beta-amyloid in the brain and the release of inflammatory cytokines. The genes APP, presenilin 1, presenilin 2, and apolipoprotein (ApoE) play a key role in Alzheimer's disease. Due to the important role of the ApoE gene in this disease, common varieties and mutations of this gene can be considered as potential biomarkers of AD. This aimed to review study was to describe the characteristics of Alzheimer's disease and also to introduce the ApoE gene as a biomarker of this disease.

10.22034/CAJMPSI.2021.05.01 **E-ISSN: 2783-0993**

*Corresponding author: mohajertf@mums.ac.ir (F. Mohajertehran)

Introduction

Alzheimer's disease (AD) is an irreversible disease and a progressive brain disorder that slowly impairs memory and thinking skills and ultimately the ability to do simple tasks. In most people with AD, symptoms first appeared in the mid-60 years old. Alzheimer's disease usually starts slowly and gets worse over time. Alzheimer's disease accounts for 60 to 70 percent of all dementia cases and is the third leading cause of death in the United States. Alzheimer's disease is characterized by the accumulation of amyloid plaques and neuronal fibers in the brain (1, 2). The most common early symptom is difficulty remembering recent events (short-term memory loss). As the disease progresses, symptoms can include language problems, confusion (including getting lost easily), mood swings, loss of mobility, loss of self-care management, and behavioral problems. The most common early symptom is difficulty remembering recent events (short-term memory loss). As the disease progresses, symptoms can include language problems, confusion (including getting lost easily), mood swings, loss of mobility, loss of self-care management, and behavioral problems (3). Following the decline of individual circumstances, they are often excluded from family and society. Gradually, bodily functions are lost and eventually lead to death. Although the rate of disease progression can vary, the average life expectancy from the time of diagnosis is between three and nine years (4). The prevalence of Alzheimer's disease is projected to reach 115 million by 2050, posing a serious threat to the health of older people and a significant economic burden on communities (2).

Alzheimer's disease is classified into two familial types and sporadic. Family type is the time when another family member has already had the disease, and approximately 25% of this disease is family type. The remaining 75% of patients are of the sporadic types who have no history of the disease in close family members (5). Alzheimer's disease is also divided into the early or late onset of symptoms, with early-onset before age 65 and late-onset after this age. Almost all Alzheimer's cases are sporadic and about 90% of the familial type appear late and only in less than 10% of this type of disease symptoms appear at a younger age (6). Except for the age of onset of the disease and the existence of family history, no difference has been observed in the pathological characteristics of the two types of familial and sporadic diseases (7).

The most important pathophysiological features of Alzheimer's disease include dysfunction of the cholinergic system, increased oxidative stress, inflammation, and death of nerve cells, reduction of nervous synapses, cerebral atrophy, decreased steroid hormones, and neurotoxicity induced by glutamate neurotransmitter (8). Alzheimer's disease has two major neuropathologic characteristics: 1) the accumulation of beta-amyloid plaques in the extracellular peptides neurons that constitute the main component of the plaques, and 2) The formation of neurofibrillary tangles within neurons is caused by hyperphosphorylation of Tau proteins seen in the hippocampus and other cortical areas (9).

Many environmental and genetic factors play a role in increasing the risk of Alzheimer's disease. Environmental factors such as heavy metals, air pollution, insecticides, nutrition status, oxidative stress, etc. can change the risk of developing this disease (10, 11). Genetic factors such as mutations and genetic polymorphisms can also increase the risk of Alzheimer's disease. In the familial type, mutations have been reported in PSEN1, PSEN2, and APP genes (12, 13). Mutations in these three genes are rarely seen sporadically. The association of the sporadic type with the mutation of the APOE.e4 allele has been greater, increasing the risk of Alzheimer's by up to three times. Alzheimer's has also recently been linked to rare genes such as TERM2, SORL1, and ABC7. This study aimed to narrate the characteristics of this disease and introduction of apolipoprotein E gene as its genetic biomarker (14, 15).

Signs and symptoms of Alzheimer's disease in different stages

Pre-dementia stage

The first symptoms are often mistakenly attributed to aging or stress (16). These early symptoms can affect the most complex activities of a person's daily life (17). The most significant problem is short-term memory loss, which is a problem in remembering recent facts and the inability to obtain new information (18, 19).

The early-stage

In people with Alzheimer's disease, an increase in learning and memory impairment eventually leads to a definitive diagnosis. In a small percentage of cases, language problems, executive activities, agnosia, or apraxia are more prominent than memory problems (20). Alzheimer's disease does not affect all memory capacity equally. Older memories of a person's life (episodic memory), learning facts (semantic memory), and implicit memory (memory of how things are done, such as using a fork to eat or drinking from a glass) is less affected than new facts or memories (21, 22).

Language problems are mainly characterized by a decrease in vocabulary and a decrease in the eloquence of words, which leads to an obvious decrease in written and spoken language. At this stage, the person with Alzheimer's is usually able to communicate sufficiently in basic ideas (20, 23). While the proper performance of movement tasks such as writing, drawing, or dressing, coordinating specific movements and controlling the execution of movements (apraxia) may be present, but they are not usually considered. As the disease progresses, people with Alzheimer's disease can often perform many tasks independently but may need more help or supervision (20).

Middle stage

Progressive dementia eventually prevents the patient from performing the most common activities of daily living independently. Speech difficulties due to the inability to remember words appear as a result of incorrect use of alternative words. Reading and writing skills are also lost. Coordination of complex activities decreases over time as Alzheimer's disease progresses, increasing the risk of falling. At this stage, memory problems increase and the person may not be able to recognize close relatives, and long-term memory that was previously healthy is impaired. Behavioral and neurological changes are becoming more common. Common symptoms such as dizziness, mood swings, and emotional distress that lead to crying or resistance to care increase. About 30% of people with Alzheimer's disease show symptoms of hallucinations. Patients also lose understanding of the limitations and course of their disease and urinary incontinence increases (20).

Advanced stage

During the final stages, the patient is completely dependent on the nurse. Speech is limited to simple words or even single words and eventually leads to a complete loss of speech ability. Despite the loss of verbal language skills, people often can understand and respond to emotional signals. Although aggression can still be present, severe indifference and fatigue are much more common symptoms of this stage of the disease. People with Alzheimer's disease are ultimately unable to perform even the simplest tasks independently. Muscle strength and mobility are lost until the person is unable to feed and get up. The cause of death is usually an external factor such as wound infection or pneumonia, not the disease itself (20).

Cause of Alzheimer's disease

The cause of most cases of Alzheimer's disease is not yet fully understood, except in 1 to 5 percent of cases where genetic differences are identified (24). Alzheimer's disease is a complex disease and is influenced by the environment and genetic and epigenetic factors. In people with early-onset Alzheimer's disease, the cause of the disease is usually a genetic mutation. Late-onset Alzheimer's disease is caused by a series of complex changes in the brain that occur over decades. The importance of each of these factors in increasing or decreasing the risk of Alzheimer's disease may vary from person to person (1, 25-27). Some risk factors of Alzheimer's disease are illustrated in Figure 1.

Environmental factors and lifestyle

Research suggests several factors beyond the genetic factors involved in the development and spread of Alzheimer's disease. Risk factors for cardiovascular disease such as high cholesterol, high blood pressure,

diabetes, and smoking are associated with the risk of incidence and developing Alzheimer's disease (28, 29). Mental activities such as reading, playing mind games, completing crossword puzzles, playing musical instruments, and regular social interaction reduce the risk of Alzheimer's disease (30). People on a diet high in saturated fats and simple carbohydrates are at higher risk for Alzheimer's disease (31). But evidence shows that foods rich in flavonoids such as cocoa and tea (32, 33) as well as healthy foods, the Mediterranean and Japanese diet (34) and caffeine (35) reduce the risk of Alzheimer's disease.

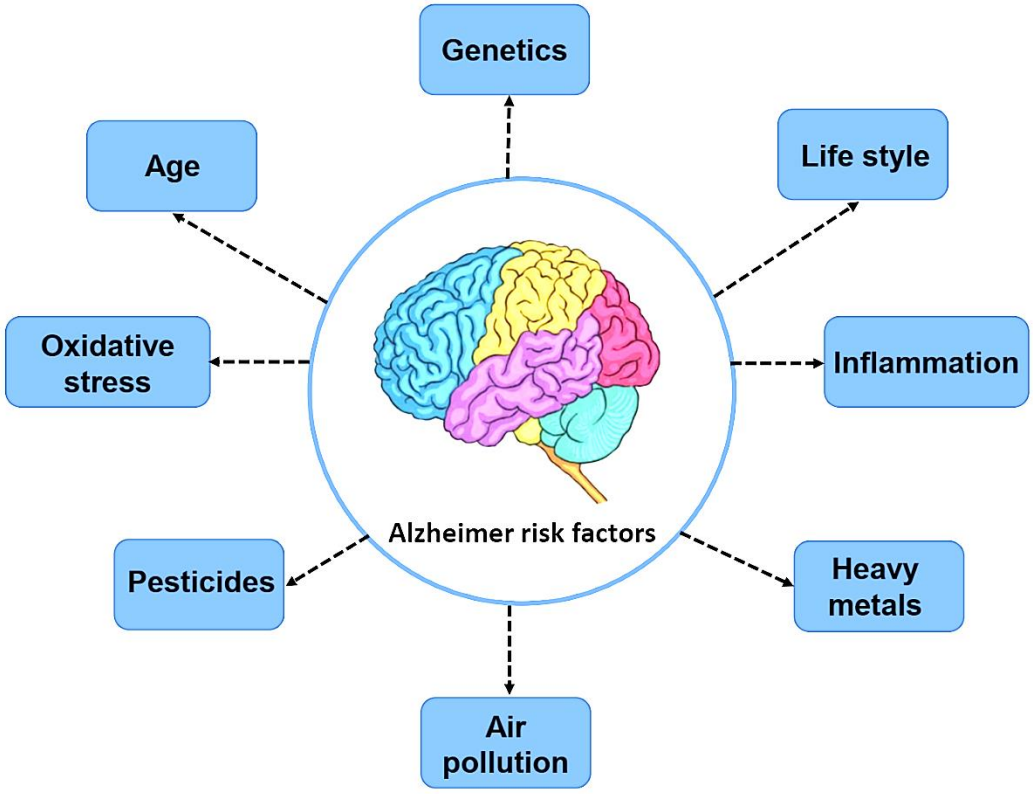


Figure 1. Some common risk factors of AD. Environmental and genetic are two common categories of risk factors for Alzheimer's disease.

Role of oxidative stress in Alzheimer's disease

In the brain tissue of Alzheimer's patients as well as healthy elderly people, mitochondrial dysfunction can lead to the release of oxidizing free radicals and oxidative damage. Oxidative stress markers can even be seen earlier than pathological changes in Alzheimer's disease, and beta-amyloid peptide appears to be a major factor in the formation of these markers (36). Peptide beta-amyloid by inhibiting main mitochondrial enzymes such as cytochrome c oxidase and key enzymes in Krebs cycle such as alpha-ketoglutarate and pyruvate dehydrogenase can have toxic effects on mitochondrial function, leading to impaired electron transfer mechanism, making the ATP, and oxygen metabolism. Following these processes, the amount of free radicals of superoxide and hydrogen peroxide increases and oxidative stress is created.

Mitochondrial hydrogen peroxide diffuses into the cytoplasmic space and forms the hydroxyl radical. Activation of microglia by beta-amyloid peptide also produces high levels of nitric oxide radicals (37, 38). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) with lipid peroxidation of cell membranes and organelles membrane produce toxic substances such as hydroxynonenal and malondialdehyde (MDA) (39). Lipid peroxidation of the cell membrane also promotes phosphorylation of tau protein and its accumulation in order to form neurofibrillary tangle. Oxidative damage can increase the permeability of cell membranes to calcium ions and other divalent ions cause the accumulation of calcium inside the nerve cell, thereby leading to

neurotoxicity and consequently dysfunction (40). In Alzheimer's disease, beta-amyloid peptides inside the nerve cell can break down mitochondrial DNA sequences, thereby reducing the number of synapses. Changes in mitochondrial membrane potential in this disease can also activate caspase enzymes. Exposure to beta-amyloid peptides also activates JNK enzymes and protein kinases p38 and p53, which are associated with the phenomenon of apoptosis. The beta-amyloid peptide can also inhibit glucose transport across cell membranes through the mechanism of inhibition of attachment of vesicle containing glucose-transporter 3 (GluT3) to membrane and its placement in membranes (41, 42). Decreased ATP production due to intracellular glucose deficiency and transmission damage can worsen the condition and may lead to cell death and a decrease in the number of neurons in Alzheimer's disease (43, 44).

Inflammation involvement in the AD

Accumulation of beta-amyloid peptides in the brain activates astrocytes and microglia and releases biochemical factors in the brains of Alzheimer's patients. These cells activate beta-amyloid peptides by their activity and degrade them to remove them from the environment (45, 46). Chronic exposure to this peptide releases chemokines and some damaging cytokines such as interleukin 1-beta, interleukin-6, interleukin-8, tumor necrosis factor-alpha, and macrophage inflammatory protein-1 alpha (47). Interleukin-1 alpha, as well as interleukin-1 beta, is important proinflammatory cytokines in the brains of Alzheimer's patients (48).

Interleukin-1 beta is involved in ROS production and lipid peroxidation. Elevated levels of this interleukin are associated with increased activity of protein kinase-activating enzymes with mitogen, p38, JNK, kinase, and caspase-3 which are related to apoptosis and a decrease in the number of synapses (49, 50). Interleukin-1 beta also reduces the release of the acetylcholine neurotransmitter in the synaptic space, which in turn can lead to more cognitive impairment in Alzheimer's patients (51). On the other hand, the release of chemokines can increase the migration of monocytes from the bloodstream into the brain tissue and trigger inflammatory responses. This process in Alzheimer's disease can be accelerated by damage to the blood-brain barrier (52).

Genetics

Alzheimer's disease is genetically complex and heterogeneous (53, 54). Early-onset Alzheimer's disease usually occurs between the ages of 30 and 60 and affects less than 5% of all people with Alzheimer's. Most of these cases are due to a genetic mutation in the three genes APP (encoding amyloid protein), presenilin 1, and presenilin 2. This form of Alzheimer's disease is known as Early-onset familial Alzheimer's disease (FAD) (55-57). In people with late-onset Alzheimer's disease, symptoms often appear in their mid-60 years old. In late-onset Alzheimer's, the apolipoprotein E (APOE) gene is altered. One form of the apolipoprotein E gene is APOE ε4, which increases a person's risk of developing the disease and is also associated with Early-onset disease (58). Previous reports suggest that genetic polymorphisms in key genes can also be an important risk factor for Alzheimer's disease (59, 60). In this regard, a complete and accurate genome study is needed (61).

Apolipoprotein E as a genetic biomarker for Alzheimer's disease susceptibility

Apo-E has three genetic isoforms (ε2, ε3, and ε4) that are associated with the plasma levels of lipoproteins (62). Apo-E binds to the remnant receptor and the low-density lipoprotein (LDL) receptor for modulation of the triglyceride-rich lipoprotein catabolism (63). In nervous tissue, Apo-E is involved in the mobilization and rearrangement of cholesterol in growth, repair, and preservation of neuronal membranes and myelin through development or after injury (64).

The Apo-E importance is highlighted in AD by its existence within the AD plaques (65). Apo-E mRNA, which plays a crucial role in compensatory CNS development and synaptogenesis decreases in the hippocampus of AD patients (66). The Apo-E3 establishes in 50–90% of people, while Apo-E4 establishes in 5–35% of people are identified to be a risk factor of AD. In a meta-analysis, Agarwal & Tripathi 2014, studied the association of Apo-E gene polymorphisms with AD in the Indian population (65). In their study, a total of seven

eligible papers demonstrating data from 417 AD patients and 651 healthy controls were analyzed. The genotypes e2/4, e3/4, and e4/4 and allele e4 were associated with AD risk, while genotypes e2/3, e3/3, and allele e3 were found to be protective genetic factors for AD. This study suggested that all genotypes carrying allele e4 are correlated with an elevated risk of AD (67).

In addition to the variation in the coding region, the discovery of the SNPs within the *APO-E* promoter could be associated with AD risk. The APO-E gene has a complex promoter with numerous regulatory elements positioned in the 5' flanking region and the first intron of the gene (68). Therefore, variations in this sequence may have functional effects mediated by the Apo-E gene regulation. The -427 T/C (rs769446), -491 A/T (rs449647) and -219 T/G (rs405509) polymorphisms have been known at the upstream start site of the Apo-E gene. These SNPs could impact APO-E transcription (69). Though numerous studies have assessed the effect of the mentioned polymorphisms on AD susceptibility, the results remain conflicting. Recently, a meta-analysis was performed to evaluate the current status of the association between these polymorphisms and the AD risk (70).

In the mentioned meta-analysis, a total of 23 eligible papers were retrieved that included 5703 AD patients and 5692 healthy controls. The allele rs769446C revealed a significant association with increased risk of AD, whereas rs449647 and rs405509 genetic polymorphisms were not associated with increased risk of AD. It revealed that rs769446 variation in the promoter sequence of APO-E may be a risk factor for AD. Further studies with larger sample size on the role of these promoter polymorphisms of *APO-E* in AD are still awaited (70).

Some current studies have reported a correlation between AD and diabetes mellitus (DM) (71, 72). These reports established that the resistance to insulin in the CNS in some patients with AD, which contributed to impaired functions of cognitive processors. According to this assumption, some studies have investigated the efficiency of anti-diabetic factors for AD treatment (73-75). Among these factors, thiazolidinediones (TZD) showed several desirable properties in vitro and in vivo training (75-77). These factors have a role to clear Aβ via the function of Apo-E (78). Some clinical trials show that TZD could improve cognitive function in patients with AD (79-81).

Intranasal use of insulin for AD displayed enhanced cognitive function in AD patients with Apo-E4-negative genotype proposing that the treatment efficiency could be influenced by Apo-E genetic polymorphisms (73). Their finding proposes that Apo-E genetic variations may contribute to inter-individual variability in the pathogenesis of AD, and suggests an association of Apo-E gene variations to insulin sensitivity. Therefore, the therapeutic effects of TZD for AD treatment could be affected by Apo-E gene variations (Figure 2). An examination into this probability could result in recognition of the suitable population for treatment of AD, although the TZD efficacy for AD is restricted (82).

In a meta-analysis, Iketani et al., (2018) examined whether Apo-E gene variations affect the TZD efficiency for AD (81). The pooled data from three eligible randomized controlled training with a total of 2381 subjects were analyzed. Although their study should be deduced with carefulness due to the limited number of studies, their findings propose that Apo-E gene variations influence the rosiglitazone efficiency for AD patients. This outcome could provide beneficial evidence for the development of new agents for AD (83).

Apo-E gene modulations and Apo-E features are hopeful targets for the development of drug and AD therapy. APO-E gene delivery could be considered as a promising plan to regulate the levels of brain Apo-E. Gene delivery of APO-E by adeno-associated virus (AAV) into the brains of the amyloid mice model verified APO-E isoform-dependent influences on the pathology of the Aβ. Precisely, Apo-E2 expression via AAV, even in a low level (~15% of the endogenous Apo-E), might decrease the Aβ levels in the brain, though Apo-E4 expression (~10% of the endogenous Apo-E) impaired synaptic loss and the Aβ accumulation/deposition in amyloid mice models (84, 85).

Similarly, opposing influences of the mentioned Apo-E gene carrier on the metabolism of mouse endogenous Aβ were described in Apo-E targeted-replacement (TR) mice lack of amyloid background (86). In addition, a true reduction of Aβ42 levels and Aβ deposition was detected when a lentiviral vector was used to

the expression of Apo-E2 in the amyloid mice model (87). Overall, these findings show that an increase of Apo-E2 by gene therapy, but not Apo-E4, could be useful in AD pathogenesis regardless of Aβ pathology.

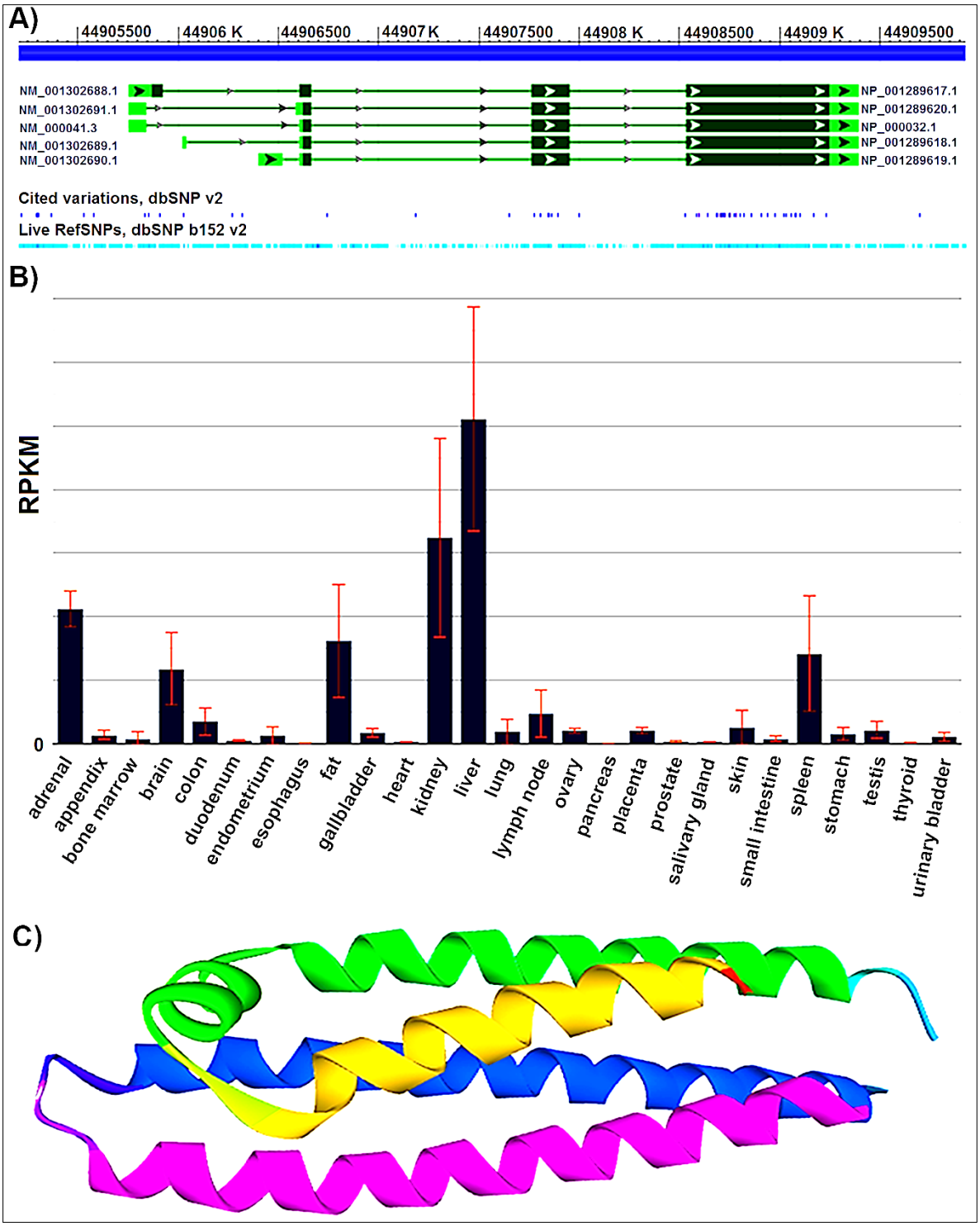


Figure 2. Gene map, tissue expression profile and three-dimensional structure of Apo-E. A) The Apo-E with chromosomal location 19q13.32 consisted of 6 exons. B) This gene expresses in different tissues including the brain at a relatively high level. C) The 3D structure of Apo-E displays four alpha-helix regions for this protein.

For clinical use of targeted Apo-E gene therapy, the therapeutic outcome of gene delivery with viral-mediated Apo-E requires to be experimentally specified in amyloid model mice carrying different isoforms of human Apo-E. For instance, targeted application of AAV expressing Apo-E under the regulation of promoter of GFAP via stereotactic injection to astrocytes in the pathology-prone regions of the hippocampus or frontal lobe

of amyloid mice model (86) could provide suitable in vivo pre-clinical evaluation. With regard to carriers of Apo-E e4, gene silencing methods using interfere RNA and antisense oligonucleotide for Apo-E could display better therapeutic efficiency than the gene overexpression, while further investigations are necessary (88, 91). However, there are some papers investigating the role of apoptosis in the pathology of stroke which can help in better understanding these contents (92-95).

Conclusion

Alzheimer's disease is one of the most common age-related neurodegenerative diseases characterized by the extracellular accumulation of beta-amyloid plaques, neurofibrillary tangle, and hyperphosphorylated tau. Therefore, Aβ is an important molecule in the pathogenesis of Alzheimer's disease and can play a role in interfering with a mitochondrial function such as lack of energy metabolism, production of reactive oxygen species, etc. Aβ gradually accumulates in the matrix of the mitochondria and is directly related to mitochondrial toxicity. Mitochondria are an important organ in the production of ROS and are associated with oxidative stress with neuronal death and neurological dysfunction, indicating a key pathogenic role of oxidative stress in Alzheimer's disease. Both microglia and astrocytes have been shown to produce beta-amyloid protein as one of the major pathological features of AD. Aβ itself has been shown to activate many inflammatory components as a proinflammatory agent. This protein can also cause the production of inflammatory cytokines in the brain. Genetic factors also play a key role in increasing the risk of Alzheimer's disease. Known genes play a role in Alzheimer's disease, one of the most important of which is apolipoprotein E. Therefore, common mutations in this gene can be considered as potential biomarkers of this disease.

References

1. Ebbert MT, Boehme KL, Wadsworth ME, Staley LA, Mukherjee S, Crane PK, Ridge PG, Kauwe JS. Alzheimer's Disease Neuroimaging Initiative, Alzheimer's Disease Genetics Consortium. Interaction between variants in CLU and MS4A4E modulates Alzheimer's disease risk. Alzheimer's Dement 2016; 12(2): 121-129. https://doi.org/10.1016/j.jalz.2015.08.163
2. Ebbert MT, Ridge PG, Wilson AR, Sharp AR, Bailey M, Norton MC, Tschanz JT, Munger RG, Corcoran CD, Kauwe JS. Population-based analysis of Alzheimer's disease risk alleles implicates genetic interactions. Biol Psychiatry 2014; 75(9): 732-737. https://doi.org/10.1016/j.biopsych.2013.07.008
3. Sparks MB. Inpatient care for persons with Alzheimer's disease. Alzheimer's Care Today 2008; 9(3): 204-210. https://doi.org/10.1097/01.CNQ.0000306399.09777.24
4. Cracco L, Appleby BS, Gambetti P. Fatal familial insomnia and sporadic fatal insomnia. Handb Clin Neurol 2018; 153: 271-299. https://doi.org/10.1016/B978-0-444-63945-5.00015-5
5. Davidson YS, Raby S, Foulds PG, Robinson A, Thompson JC, Sikkink S, Yusuf I, Amin H, DuPlessis D, Troakes C, Al-Sarraj S. TDP-43 pathological changes in early onset familial and sporadic Alzheimer's disease, late onset Alzheimer's disease and Down's syndrome: association with age, hippocampal sclerosis and clinical phenotype. Acta neuropathol 2011; 122(6): 703-713. https://doi.org/10.1007/s00401-011-0879-y
6. Ryan NS, Rossor MN. Correlating familial Alzheimer's disease gene mutations with clinical phenotype. Biomark Med 2010; 4(1): 99-112. https://doi.org/10.2217/bmm.09.92
7. Shah RS, Lee H-G, Xiongwei Z, Perry G, Smith MA, Castellani RJ. Current approaches in the treatment of Alzheimer's disease. Biomed Pharmacother 2008; 62(4): 199-207. https://doi.org/10.1016/j.biopha.2008.02.005
8. Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. Acta Neuropathol 2009; 118(1): 5-36. https://doi.org/10.1007/s00401-009-0532-1
9. Agnihotri A, Aruoma OI. Alzheimer's disease and Parkinson's disease: a nutritional toxicology perspective of the impact of oxidative stress, mitochondrial dysfunction, nutrigenomics and environmental chemicals. J Am Coll Nutr 2020; 39(1): 16-27. https://doi.org/10.1080/07315724.2019.1683379
10. Tanzi RE, Bertram L. New frontiers in Alzheimer's disease genetics. Neuron 2001; 32(2): 181-184. https://doi.org/10.1016/S0896-6273(01)00476-7

11. Bagyinszky E, Youn YC, An SSA, Kim S. The genetics of Alzheimer's disease. Clin Interv Aging 2014; 9: 535. https://doi.org/10.2147/CIA.S51571
12. Sandbrink R, Hartmann T, Masters C, Beyreuther K. Genes contributing to Alzheimer's disease. Mol Psychiatry 1996; 1(1): 27-40.
13. Guerreiro RJ, Gustafson DR, Hardy J. The genetic architecture of Alzheimer's disease: beyond APP, PSENs and APOE. Neurobiol Aging 2012; 33(3): 437-456. https://doi.org/10.1016/j.neurobiolaging.2010.03.025
14. Waldemar G, Dubois B, Emre M, Georges J, McKeith IG, Rossor M, Scheltens P, Tariska P, Winblad B. Recommendations for the diagnosis and management of Alzheimer's disease and other disorders associated with dementia: EFNS guideline. Eur J Neurol 2007; 14(1): e1-e26. https://doi.org/10.1111/j.1468-1331.2006.01605.x
15. Nygård L. Instrumental activities of daily living: a stepping-stone towards Alzheimer's disease diagnosis in subjects with mild cognitive impairment? Acta Neurol Scand Suppl 2003; 107: 42-46. https://doi.org/10.1034/j.1600-0404.107.s179.8.x
16. Bäckman L, Jones S, Berger AK, Laukka EJ, Small B. Multiple cognitive deficits during the transition to Alzheimer's disease. J Intern Med 2004; 256(3): 195-204. https://doi.org/10.1111/j.1365-2796.2004.01386.x
17. Landes AM, Sperry SD, Strauss ME, Geldmacher DS. Apathy in Alzheimer's disease. J Am Geriatr Soc 2001; 49(12): 1700-1707. https://doi.org/10.1046/j.1532-5415.2001.49282.x
18. Förstl H, Kurz A. Clinical features of Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci 1999; 249(6): 288-290. https://doi.org/10.1007/s004060050101
19. Jelicic M, Bonebakker AE, Bonke B. Implicit memory performance of patients with Alzheimer's disease: a brief review. Int Psychogeriatr 1995; 7(3): 385-392. https://doi.org/10.1017/S1041610295002134
20. Carlesimo GA, Oscar-Berman M. Memory deficits in Alzheimer's patients: a comprehensive review. Neuropsychol Rev 1992; 3(2): 119-169. https://doi.org/10.1007/BF01108841
21. Taler V, Phillips NA. Language performance in Alzheimer's disease and mild cognitive impairment: a comparative review. J Clin Exp Neuropsychol 2008; 30(5): 501-556. https://doi.org/10.1080/13803390701550128
22. Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol 2014; 88(4): 640-651. https://doi.org/10.1016/j.bcp.2013.12.024
23. Bullock JM, Medway C, Cortina-Borja M, Turton JC, Prince JA, Ibrahim-Verbaas CA, Schuur M, Breteler MM, van Duijn CM, Kehoe PG, Barber R. Discovery by the Epistasis Project of an epistatic interaction between the GSTM3 gene and the HHEX/IDE/KIF11 locus in the risk of Alzheimer's disease. Neurobiol Aging 2013; 34(4): e1-e7. https://doi.org/10.1016/j.neurobiolaging.2012.08.010
24. Combarros O, Cortina-Borja M, Smith AD, Lehmann DJ. Epistasis in sporadic Alzheimer's disease. Neurobiol Aging 2009; 30(9): 1333-1349. https://doi.org/10.1016/j.neurobiolaging.2007.11.027
25. Kauwe JS, Bertelsen S, Mayo K, Cruchaga C, Abraham R, Hollingworth P, Harold D, Owen MJ, Williams J, Lovestone S, Morris JC. Suggestive synergy between genetic variants in TF and HFE as risk factors for Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet 2010; 153(4): 955-959. https://doi.org/10.1002/ajmg.b.31053
26. Patterson C, Feightner JW, Garcia A, Hsiung G-YR, MacKnight C, Sadovnick AD. Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease. CMAJ 2008; 178(5): 548-556. https://doi.org/10.1503/cmaj.070796
27. Rosendorff C, Beeri MS, Silverman JM. Cardiovascular risk factors for Alzheimer's disease. Am J Geriatr Cardiol 2007; 16(3): 143-149. https://doi.org/10.1111/j.1076-7460.2007.06696.x
28. Stern Y. Cognitive reserve and Alzheimer disease. Alzheimer Dis Assoc Disord 2006; 20: S69-S74. https://doi.org/10.1097/01.wad.0000213815.20177.19
29. Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav 2011; 103(1): 59-68. https://doi.org/10.1016/j.physbeh.2010.12.003

30. Nehlig A. The neuroprotective effects of cocoa flavanol and its influence on cognitive performance. Br J Clin Pharmacol 2013; 75(3): 716-727. https://doi.org/10.1111/j.1365-2125.2012.04378.x
31. Stoclet J-C, Schini-Kerth V. Flavonoides alimentaires et santé humaine. Ann Pharm Fr 2011; 69(2): 78-90. https://doi.org/10.1016/j.pharma.2010.11.004
32. Hu N, Yu JT, Tan L, Wang YL, Sun L, Tan L. Nutrition and the risk of Alzheimer's disease. BioMed Res Int 2013; 2013. https://doi.org/10.1155/2013/524820
33. Santos C, Costa J, Santos J, Vaz-Carneiro A, Lunet N. Caffeine intake and dementia: systematic review and meta-analysis. J Alzheimer's Dis 2010; 20(s1): S187-S204. https://doi.org/10.3233/JAD-2010-091387
34. Gella A, Durany N. Oxidative stress in Alzheimer disease. Cell Adh Migr 2009; 3(1): 88-93. https://doi.org/10.4161/cam.3.1.7402
35. Gibson G, Sheu KF, Blass J. Abnormalities of mitochondrial enzymes in Alzheimer disease. J Neural Transm 1998; 105(8): 855-870. https://doi.org/10.1007/s007020050099
36. Pagani L, Eckert A. Amyloid-Beta interaction with mitochondria. Int J Alzheimer's Dis 2011; 2011. https://doi.org/10.4061/2011/925050
37. Repetto M, Semprine J, Boveris A. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. Lipid Peroxid 2012; 1: 3-30. https://doi.org/10.5772/45943
38. Liu Q, Smith MA, Avilá J, DeBernardis J, Kansal M, Takeda A, Zhu X, Nunomura A, Honda K, Moreira PI, Oliveira CR. Alzheimer-specific epitopes of tau represent lipid peroxidation-induced conformations. Free Radic Biol Med 2005; 38(6): 746-754. https://doi.org/10.1016/j.freeradbiomed.2004.11.005
39. Eckert A, Keil U, Marques CA, Bonert A, Frey C, Schüssel K, Müller WE. Mitochondrial dysfunction, apoptotic cell death, and Alzheimer's disease. Biochem Pharmacol 2003; 66(8): 1627-1634. https://doi.org/10.1016/S0006-2952(03)00534-3
40. Galindo MF, Ikuta I, Zhu X, Casadesus G, Jordán J. Mitochondrial biology in Alzheimer's disease pathogenesis. J Neurochem 2010; 114(4): 933-945. https://doi.org/10.1111/j.1471-4159.2010.06814.x
41. Poh Loh K, Hong Huang S, De Silva R, H Tan BK, Zhun Zhu Y. Oxidative stress: apoptosis in neuronal injury. Curr Alzheimer Res 2006; 3(4): 327-337. https://doi.org/10.2174/156720506778249515
42. Münch G, Schinzel R, Loske C, Wong A, Durany N, Li JJ, Vlassara H, Smith MA, Perry G, Riederer P. Alzheimer's disease–synergistic effects of glucose deficit, oxidative stress and advanced glycation endproducts. J Neural Transm 1998; 105(4-5): 439-461. https://doi.org/10.1007/s007020050069
43. Agostinho P, A Cunha R, Oliveira C. Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Des 2010; 16(25): 2766-2778. https://doi.org/10.2174/138161210793176572
44. Majumdar A, Cruz D, Asamoah N, Buxbaum A, Sohar I, Lobel P, Maxfield FR. Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils. Mol Biol Cell 2007; 18(4): 1490-1496. https://doi.org/10.1091/mbc.e06-10-0975
45. Domingues C, AB da Cruz e Silva O, Henriques A. Impact of cytokines and chemokines on Alzheimer's disease neuropathological hallmarks. Curr Alzheimer Res 2017; 14(8): 870-882. https://doi.org/10.2174/1567205014666170317113606
46. Shaftel SS, Griffin WST, O'Banion MK. The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. J Neuroinflamm 2008; 5(1): 1-12. https://doi.org/10.1186/1742-2094-5-7
47. Kim JM, Lee U, Kang JY, Park SK, Shin EJ, Kim HJ, Kim CW, Kim MJ, Heo HJ. Anti-Amnesic Effect of Walnut via the Regulation of BBB Function and Neuro-Inflammation in Aβ1-42-Induced Mice. Antioxidants 2020; 9(10): 976. https://doi.org/10.3390/antiox9100976
48. Hossain MF, Uddin MS, Uddin GS, Sumsuzzman DM, Islam MS, Barreto GE, Mathew B, Ashraf GM. Melatonin in Alzheimer's disease: a latent endogenous regulator of neurogenesis to mitigate Alzheimer's neuropathology. Mol Neurobiol 2019; 56(12): 8255-8276. https://doi.org/10.1007/s12035-019-01660-3
49. H Ferreira-Vieira T, M Guimaraes I, R Silva F, M Ribeiro F. Alzheimer's disease: targeting the cholinergic system. Curr Neuropharmacol 2016; 14(1): 101-115. https://doi.org/10.2174/1570159x13666150716165726

50. Cai Z, Hussain MD, Yan LJ. Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. Int J Neurosci 2014; 124(5): 307-321. https://doi.org/10.3109/00207454.2013.833510
51. Chan G, White CC, Winn PA, Cimpean M, Replogle JM, Glick LR, Cuerdon NE, Ryan KJ, Johnson KA, Schneider JA, Bennett DA. CD33 modulates TREM2: convergence of Alzheimer loci. Nat Neurosci 2015; 18(11): 1556-1558. https://doi.org/10.1038/nn.4126
52. Ertekin-Taner N. Genetics of Alzheimer's disease: a centennial review. Neurol Clin 2007; 25(3): 611-667. https://doi.org/10.1016/j.ncl.2007.03.009
53. Wilson RS, Barral S, Lee JH, Leurgans SE, Foroud TM, Sweet RA, Graff-Radford N, Bird TD, Mayeux R, Bennett DA. Heritability of different forms of memory in the Late Onset Alzheimer's Disease Family Study. J Alzheimer's Dis 2011; 23(2): 249-255. https://doi.org/10.3233/JAD-2010-101515
54. Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, Fiske A, Pedersen NL. Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 2006; 63(2): 168-174. https://doi.org/10.1001/archpsyc.63.2.168
55. Waring SC, Rosenberg RN. Genome-wide association studies in Alzheimer disease. Arch Neurol 2008; 65(3): 329-334. https://doi.org/10.1001/archneur.65.3.329
56. Zhu L, Zhong M, Elder GA, Sano M, Holtzman DM, Gandy S, Cardozo C, Haroutunian V, Robakis NK, Cai D. Phospholipid dysregulation contributes to ApoE4-associated cognitive deficits in Alzheimer's disease pathogenesis. Proc Natl Acad Sci 2015; 112(38): 11965-11970. https://doi.org/10.1073/pnas.1510011112
57. Hua Y, Zhao H, Kong Y, Ye M. Association between the MTHFR gene and Alzheimer's disease: a meta-analysis. Int J Neurosci 2011; 121(8): 462-471. https://doi.org/10.3109/00207454.2011.578778
58. Bosco P, Guéant-Rodríguez RM, Anello G, Romano A, Namour B, Spada RS, Caraci F, Tringali G, Ferri R, Guéant JL. Association of IL-1 RN* 2 allele and methionine synthase 2756 AA genotype with dementia severity of sporadic Alzheimer's disease. J Neurol Neurosurg Psychiatry 2004; 75(7): 1036-1038. http://dx.doi.org/10.1136/jnnp.2003.025866
59. Kazemi E, Zargooshi J, Kaboudi M, Heidari P, Kahrizi D, Mahaki B, Mohammadian Y, Khazaei H, Ahmed K. A genome-wide association study to identify candidate genes for erectile dysfunction. Brief Bioinform 2021; 22(4): bbaa338. https://doi.org/10.1093/bib/bbaa338
60. Rasmussen KL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. Ann Neurol 2015; 77(2): 301-311. https://doi.org/10.1002/ana.24326
61. Moriarty PM, Varvel SA, Gordts PL, McConnell JP, Tsimikas S. Lipoprotein (a) mass levels increase significantly according to APOE genotype: an analysis of 431 239 patients. Arterioscler Thromb Vasc Biol 2017; 37(3): 580-588. https://doi.org/10.1161/ATVBAHA.116.308704
62. Lane-Donovan C, Herz J. ApoE, ApoE receptors, and the synapse in Alzheimer's disease. Trends Endocrinol Metab 2017; 28(4): 273-284. https://doi.org/10.1016/j.tem.2016.12.001
63. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015; 14(4): 388-405. https://doi.org/10.1016/S1474-4422(15)70016-5
64. Theendakara V, Peters-Libeu CA, Bredesen DE, Rao RV. Transcriptional effects of ApoE4: relevance to Alzheimer's disease. Mol Neurobiol 2018; 55(6): 5243-5254. https://doi.org/10.1007/s12035-017-0757-2
65. Agarwal R, Tripathi CB. Association of apolipoprotein E genetic variation in Alzheimer's disease in Indian population: a meta-analysis. Am J Alzheimers Dis Other Demen 2014; 29(7): 575-582. https://doi.org/10.1177/1533317514531443
66. Berg DT, Calnek DS, Grinnell BW. The Human Apolipoprotein E Gene Is Negatively Regulated in Human Liver HepG2 Cells by the Transcription Factor BEF-1. J Biol Chem 1995; 270(26): 15447-15450. https://doi.org/10.1074/jbc.270.26.15447

67. Sala Frigerio C, De Strooper B. Alzheimer's disease mechanisms and emerging roads to novel therapeutics. Ann Rev Neurosci 2016; 39: 57-79. https://doi.org/10.1146/annurev-neuro-070815-014015
68. Xiao H, Gao Y, Liu L, Li Y. Association between polymorphisms in the promoter region of the apolipoprotein E (APOE) gene and Alzheimer's disease: A meta-analysis. EXCLI J. 2017;16:921. https://doi.org/10.17179/excli2017-289
69. De Felice FG. Alzheimer's disease and insulin resistance: translating basic science into clinical applications. J Clin Invest 2013; 123(2): 531-539. https://doi.org/10.1172/JCI64595
70. Hokama M, Oka S, Leon J, Ninomiya T, Honda H, Sasaki K, Iwaki T, Ohara T, Sasaki T, LaFerla FM, Kiyohara Y. Altered expression of diabetes-related genes in Alzheimer's disease brains: the Hisayama study. Cereb Cortex 2014; 24(9): 2476-2488. https://doi.org/10.1093/cercor/bht101
71. Reger MA, Watson G, Green PS, Baker LD, Cholerton B, Fishel MA, Plymate SR, Cherrier MM, Schellenberg GD, Frey Ii WH, Craft S. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-β in memory-impaired older adults. J Alzheimer's Dis 2008; 13(3): 323-331. https://doi.org/10.3233/JAD-2008-13309
72. M de la Monte S. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. Curr Alzheimer Res 2012; 9(1): 35-66. https://doi.org/10.2174/156720512799015037
73. Landreth G, Jiang Q, Mandrekar S, Heneka M. PPARγ agonists as therapeutics for the treatment of Alzheimer's disease. Neurotherapeutics 2008; 5(3): 481-489. https://doi.org/10.1016/j.nurt.2008.05.003
74. Miller BW, Willett KC, Desilets AR. Rosiglitazone and pioglitazone for the treatment of Alzheimer's disease. Ann Pharmacother 2011; 45(11): 1416-1424. https://doi.org/10.1345/aph.1Q238
75. Pipatpiboon N, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. PPARγ agonist improves neuronal insulin receptor function in hippocampus and brain mitochondria function in rats with insulin resistance induced by long term high-fat diets. Endocrinology 2012; 153(1): 329-338.https://doi.org/10.1210/en.2011-1502
76. Mandrekar-Colucci S, Karlo JC, Landreth GE. Mechanisms underlying the rapid peroxisome proliferator-activated receptor-γ-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. J Neurosci 2012; 32(30): 10117-10128. https://doi.org/10.1523/JNEUROSCI.5268-11.2012
77. Hanyu H, Sato T, Sakurai H, Iwamoto T. The role of tumor necrosis factor-alpha in cognitive improvement after peroxisome proliferator-activator receptor gamma agonist pioglitazone treatment in alzheimer's disease. J Am Geriatr Soc 2010; 58(5): 1000-1001. https://doi.org/10.1111/j.1532-5415.2010.02841.x
78. Hanyu H, Sato T, Kiuchi A, Sakurai H, Iwamoto T. Pioglitazone improved cognition in a pilot study on patients with Alzheimer's disease and mild cognitive impairment with diabetes mellitus. J Am Geriatr Soc 2009; 57(1): 177-179. https://doi.org/10.1111/j.1532-5415.2009.02067.x
79. Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease. Neurobiol Aging 2011; 32(9): 1626-1633. https://doi.org/10.1016/j.neurobiolaging.2009.10.009
80. Gold M, Alderton C, Zvartau-Hind M, Egginton S, Saunders AM, Irizarry M, Craft S, Landreth G, Linnamägi Ü, Sawchak S. Rosiglitazone monotherapy in mild-to-moderate Alzheimer's disease: results from a randomized, double-blind, placebo-controlled phase III study. Dement Geriatr Cogn Disord 2010; 30(2): 131-146. https://doi.org/10.1159/000318845
81. Iketani R, Ohno K, Kawasaki Y, Matsumoto K, Yamada H, Kishino S. Apolipoprotein E gene polymorphisms affect the efficacy of thiazolidinediones for Alzheimer's disease: a systematic review and meta-analysis. Biolo Pharm Bull 2018; 41(7): 1017-1023. https://doi.org/10.1248/bpb.b17-00929
82. Hudry E, Dashkoff J, Roe AD, Takeda S, Koffie RM, Hashimoto T, Scheel M, Spires-Jones T, Arbel-Ornath M, Betensky R, Davidson BL. Gene transfer of human Apoe isoforms results in differential modulation of amyloid deposition and neurotoxicity in mouse brain. Sci Transl Med 2013; 5(212): 212ra161-212ra161. https://doi.org/10.1126/scitranslmed.3007000

83. Cheng H, Shang Y, Jiang L, Shi Tl, Wang L. The peroxisome proliferators activated receptor-gamma agonists as therapeutics for the treatment of Alzheimer's disease and mild-to-moderate Alzheimer's disease: a meta-analysis. IntJ Neurosci 2016; 126(4): 299-307. https://doi.org/10.3109/00207454.2015.1015722
84. Hu J, Liu CC, Chen XF, Zhang Yw, Xu H, Bu G. Opposing effects of viral mediated brain expression of apolipoprotein E2 (apoE2) and apoE4 on apoE lipidation and Aβ metabolism in apoE4-targeted replacement mice. Mol Neurodegener 2015; 10(1): 1-11. https://doi.org/10.1186/s13024-015-0001-3
85. Yang M, Abdalrahman H, Sonia U, Mohammed AI, Vestine U, Wang M, Ebadi AG, Toughani M. The application of DNA molecular markers in the study of Codonopsis species genetic variation, a review. Cell Mol Biol 2020; 66(2): 23-30. https://doi.org/10.14715/cmb/2020.66.2.3
86. Dodart JC, Marr RA, Koistinaho M, Gregersen BM, Malkani S, Verma IM, Paul SM. Gene delivery of human apolipoprotein E alters brain Aβ burden in a mouse model of Alzheimer's disease. Proc Natl Acad Sci 2005; 102(4): 1211-1216. https://doi.org/10.1073/pnas.0409072102
87. Yamazaki Y, Painter MM, Bu G, Kanekiyo T. Apolipoprotein E as a therapeutic target in Alzheimer's disease: a review of basic research and clinical evidence. CNS Drugs 2016; 30(9): 773-789. https://doi.org/10.1007/s40263-016-0361-4
88. Nilsson LN, Gografe S, Costa DA, Hughes T, Dressler D, Potter H. Use of fused circulations to investigate the role of apolipoprotein E as amyloid catalyst and peripheral sink in Alzheimer's disease. Technol Innov 2012; 14(2): 199-208. https://doi.org/10.3727/194982412X13462021398010
89. Ayton S, Portbury S, Kalinowski P, Agarwal P, Diouf I, Schneider JA, Morris MC, Bush AI. Regional brain iron associated with deterioration in Alzheimer's disease: A large cohort study and theoretical significance. Alzheimer's Dement 2021; 17(7): 1244-1256. https://doi.org/10.1002/alz.12282
90. Kotze MJ, Lückhoff HK, Brand T, Pretorius J, van Rensburg SJ. Apolipoprotein E ε-4 as a genetic determinant of Alzheimer's disease heterogeneity. Degener Neurol Neuromuscul Dis 2015; 5: 9. https://doi.org/10.2147/DNND.S41721
91. Wen L, Zhang Y, Yang B, Han F, Ebadi AG, Toughani M. Knockdown of Angiopoietin-like protein 4 suppresses the development of colorectal cancer. Cell Mol Biol 2020; 66(5): 117-124. https://doi.org/10.14715/cmb/2020.66.5.21
92. Liang Y, Zhang J, Li B, Wang J. Neuroprotective effect experimental study of cystatin c preconditioning on cerebral ischemia-reperfusion injury in mice. Acta Medica Mediterr 2021; 37(1): 317-321. https://doi.org/10.19193/0393-6384_2021_1_49
93. Muñoz SS, Garner B, Ooi L. Understanding the role of ApoE fragments in Alzheimer's disease. Neurochem Res 2019; 44(6): 1297-1305. https://doi.org/10.1007/s11064-018-2629-1
94. Keene CD, Cudaback E, Li X, Montine KS, Montine TJ. Apolipoprotein E isoforms and regulation of the innate immune response in brain of patients with Alzheimer's disease. Curr Opin Neurobiol 2011; 21(6): 920-928. https://doi.org/10.1016/j.conb.2011.08.002
95. Moon YE, Kim JY, Lee SY, Kim J, Joo MA, Park HJ. Herpes zoster myelitis, with occurrence of unusual neurologic symptoms in herpes zoster infection: successful treatment of three cases. Acta Medica Mediterr 2021; 37(1): 69-71. https://doi.org/10.19193/0393-6384_2021_1_8

How to cite this paper:
Sahebkar A, Mohajertehran F, Goloobi A. Alzheimer's disease as a neurodegenerative disorder and the apolipoprotein E gene as its genetic biomarker. Cent Asian J Med Pharm Sci Innov 2021; 1(5): 205-217.