Original Research Paper

Analysis of Plasma Amyloid-Beta and Apolipoprotein E Genotypes as Markers of Alzheimer's Disease in Healthy Korean Adults

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Abstract: The presence of the ApoE4 allele interferes with human Amyloid-Beta (Aβ) clearance and reduces the neuronal damage response, which, in turn, is associated with increased Alzheimer's Disease (AD) risk. Identifying individuals with the highest genetic risk may aid clinical AD diagnosis and prognosis. However, to the best of our knowledge, no previous study has performed blood-based analysis of ApoE polymorphisms and human Aβ in individuals undergoing health checkups. Here, we aimed to investigate ApoE genotypes and Aβ levels according to the age and sex of individuals undergoing health checkups using real-time polymerase chain reaction. For ApoE genotyping and human Aβ analysis, data from 423 human Aβ samples, within the 95% confidence interval, were analyzed. The Aβ levels increased and decreased with age in female and male individuals with the ApoE genotype E4, respectively. Our study provides age and sex-based ApoE genotype and Aβ data for healthy adults, highlighting the importance of ApoE4 and Aβ as predictors of AD risk and elucidating differences according to age and sex. Our study provides details on the genetic background of AD and thus, might aid in improving the efficiency of AD diagnosis.

Keywords: Alzheimer's Disease, ApoE, β-Amyloid, Dementia, Genetics

Introduction

Alzheimer's Disease (AD), affecting approximately 26 million people worldwide (Rasheed, 2022), is a neurodegenerative disorder characterized by widespread brain atrophy and loss of cognitive function and memory. AD is also associated with cerebrovascular changes implicated in neuronal dysfunction and neurodegeneration (Janelidze *et al*., 2018). Senile dementia imposes a huge public health burden and AD alone is the seventh leading cause of death in the United States, with treatment costs of approximately $172 billion annually (Janelidze *et al*., 2016). In South Korea, which is experiencing a rapid population aging, the trend of dementia is becoming more pronounced and the number of people aged 65 and older reached 8.5 millionin 2021 and is expected to exceed 13 million by 2030 and 19 million (accounting for about 40% of older adult population) by 2050 (Gaidai *et al*., 2023). AD is frequently accompanied by cerebrovascular disease (Jellinger and Attems, 2010) and has common risk factors such as the occurrence of Apolipoprotein E (ApoE) 4, hyperlipidemia and obesity (Janelidze *et al*., 2018). The risk of AD development is mostly (approximately 60-80%) dependent on genetic factors and more than 40 AD-related genetic risk loci have already been identified, among which the ApoE allele has the strongest association with the disease (Chen *et al*., 2022). Chen *et al*. (2022) also showed that the frequency of the ApoE4 allele is significantly higher in AD cases than in healthy controls (odds ratio: 2.847; 95% confidence interval: (2.611-3.101), p<0.001).

ApoE, a major cholesterol transporter in the brain, supports lipid transport between brain cells (Dias *et al*., 2022). ApoE has three polymorphisms (E2, E3 and E4), three homozygous forms (E2/E2, E3/E3 and E4/E4) and three allelic forms (E3/E2, E4/E2 and E4/E3) (Dias *et al*., 2022; Rasheed, 2022). The ApoE4 allele is the strongest risk factor for late and early-onset AD, especially in women and is related to cardiovascular disease development (Torres *et al*., 2022; Dias *et al*., 2022; Bonham *et al*., 2016).

The ApoE4 allele interferes with human Amyloid-Beta (Aβ) clearance and reduces the neuronal damage response associated with a higher risk of AD (Sauty and Durrleman, 2023). The presence of the ApoE4 allele increases the risk of AD development and lowers the age at disease onset in a level-dependent manner (Koutsodendris *et al*., 2022). Growing evidence suggests that ApoE4 substantially affects these pathologies and several other important cellular and molecular processes in the Central Nervous System (CNS) (Koutsodendris *et al*., 2022). Understanding the physiological role of ApoE within the CNS could help elucidate the loss-of-function and gain-of-function effects that occur in AD (Vessey *et al*., 2022).

AD is pathologically characterized by extracellular aggregation of Aβ plaques, intracellular aggregation of neurofibrillary tangles consisting of hyperphosphorylated tau protein, inflammation, cerebrovascular pathology and neuronal cell death (Zhang *et al*., 2022). Aβ is usually present in the brain as a part of the signal transduction process (Johnson and Bailey, 2003). Deposition of Aβ is the most prominent pathological change in AD and it precedes brain atrophy and cognitive impairment (Kang *et al*., 2022).

Despite advances in understanding the mechanisms underlying AD development, it remains an incurable, complex disorder with a multifaceted pathophysiology (Dias *et al*., 2022). The development of preventive and therapeutic interventions makes it increasingly important to identify clinically healthy individuals at a high risk of developing AD. Aβ deposition begins to appear before the manifestation of clinical symptoms of AD; therefore, this peptide is an important biomarker that can be used to predict disease development (Sehar *et al*., 2022).

Current therapies for AD have low effectiveness and do not alter the course of the disease. Therefore, preclinical prediction of dementia development using biomarkers is important for effective intervention and disease modification (Janelidze *et al*., 2016). Supporting biomarker information is needed to facilitate clinical trials of disease-modifying therapies for AD, which are expected to be most effective in the early and middle stages of AD (Nakamura *et al*., 2018).

In this study, ApoE genotype and human Aβ levels were analyzed according to the age and sex of individuals undergoing health checks using Enzyme-Linked Immunosorbent Assay (ELISA) and real-time Polymerase Chain Reaction (PCR) analysis. ApoE genotyping and human Aβ in healthy adults could help predict AD outcomes. Overall, this study could contribute to a comprehensive understanding of the genetic background of AD development that would help improve its diagnosis.

Materials and Methods

Study Participants and Design

From January to February 2023, data from 459 specimens (blood plasma) were collected from male and female participants aged 19-70 years undergoing health checkups at Dankook University Hospital in Cheonan, Korea. We requested U2Bio (Korea) to conduct molecular biological testing for ApoE genotyping. During the same period, 457 male and female participants aged 19-70 years undergoing health checkups at Dankook University Hospital in Cheonan in Cheonan were requested to participate in human Aβ analysis by U2Bio (Korea) and sample data were collected. From the results of ApoE genotyping and human Aβ analysis, data from 423 human Aβ samples were obtained, which were within the 95% confidence interval and were analyzed.

Ethical Approval

This study was approved by the Institutional Review Board of Dankook University, Republic of Korea (No. 2023-10-020) and was conducted following the principles of the declaration of Helsinki. The requirement for participant consent was waived because the statistical data of tests conducted by medical institutions for diagnosis did not include any personal information of the participants.

Reagent Preparation and Enzyme-Linked Immunosorbent Assay

Human Aβ (aa1-42) was quantified in blood plasma samples using commercial enzyme-linked immunosorbent assay (ELISA) (Quantikine ELISA; R and D System, MA, USA) test kits according to the manufacturer's instructions. The aa1-42 conjugates were maintained at 2-8°C during use and all other reagents and samples were placed at 20-25°C before use. The ELISA standard ranged from 0-500 pg/mL. Absorbance was recorded at 450 and 540 nm.

Nucleic Acid Extraction

The collected blood plasma samples were stored at -70°C until DNA isolation for multiplex Polymerase Chain Reaction (mPCR). According to the manufacturer's instructions, DNA for mPCR was extracted using the QIAamp DSP DNA Mini Kit (QIAGEN, Seoul, Korea) in QIAcube (QIAGEN).

Real-Time Polymerase Chain Reaction Analysis (ApoE Genotyping)

Real-time polymerase chain reaction (PCR) was performed using the ApoE Genotyping Real-Time PCR Kit (BioCore, Seoul, Korea) with the CFX96 Dx real-time PCR detection system (Bio-Rad, CA, USA) according to the manufacturer's protocol. The amplification protocol

comprised one cycle at 95°C for 5 min, 45 cycles at 95°C for 5 s and 55°C for 5 s. The threshold cycle was determined according to the manufacturer's instructions.

Statistical Analysis

The one-way analysis of variance was used for the statistical studies and performed using Prism6 (Version 6.02, March 11, 2013). A post-hoc test was also used and the results are shown as mean and standard error of the mean. Statistical significance was defined at $p < 0.05$.

Results

ApoE Genotype Statistics

The ApoE4 genotypes determined using ApoE genotyping of the 459 individuals undergoing health checkups from January to February 2023 were E2/E4 (1.7%; n = 8), E3/E4 (17.0%; n = 78) and E4/E4 (0.4%; n = 2) (Fig. 1). The proportion of ApoE genotypes of E2/E2, E2/E3 and E3/E3 was 0.7% (n = 3), 13.1% (n = 60) and 67.1% (n = 308), respectively (Fig. 1). The detection rate of the ApoE4 genotype was higher in male than in female participants.

Aβ Statistics

Among male participants, the 40-49-year group had the highest average Aβ level of 73.9 pg/mL, whereas the ≤19-year group had the lowest Aβ level of 37.5 pg/mL. Among female participants, the 50-59-year group had the highest average Aβ level of 79.0 pg/mL, whereas the ≤19-year group had the lowest level of 16.0 pg/mL.

Aβ Distribution According to ApoE Genotyping in Female Participants

Among female participants, the E3/E4 ApoE genotype individuals had the highest average Aβ level of 37.9 pg/mL, whereas the E2/E3 ApoE genotype individuals had the lowest average Aβ level of 27.3 pg/mL (Fig. 2).

Statistics Within the 95% Range

Aβ Distribution According to ApoE Genotyping

Age groups 20-29 and ≥70 years in the ≥70-year group, the mean plasma Aβ level in E3/E4 ApoE genotype individuals was the highest at 83.6 pg/mL and in E2/E3 ApoE genotype individuals, the Aβ level was the lowest at 29.3 pg/mL (Fig. 1).

In the ≤19-year group, the mean plasma Aβ level in male participants with E3/E4 ApoE genotypes was the highest at 169.9 pg/mL (Fig. 1) and the Aβ level in individuals with E2/E3 ApoE genotypes was the lowest at 28.3 pg/mL. Among female participants aged ≥70 years, the mean plasma Aβ level in E3/E4 ApoE genotype individuals was the highest at 136.1 pg/mL. Among

female participants aged ≤19 years, the Aβ level in E3/E4 ApoE genotype individuals was the lowest at 22.1 pg/mL (Fig. 1). The highest Aβ levels were recorded in E3/E4 ApoE genotypes in both male and female participants, although in different age groups.
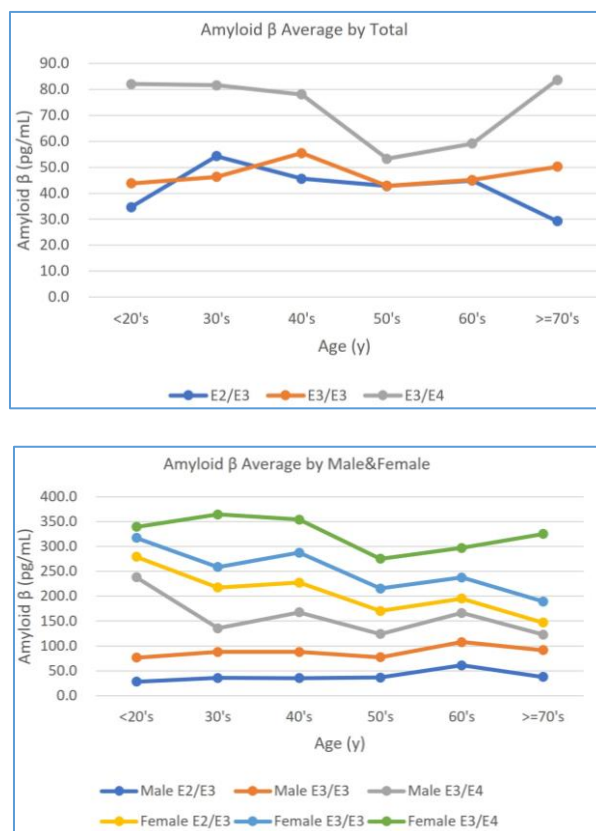
Fig. 1: Total, male and female apolipoprotein E genotypes and amyloid β distribution according to the sex and age of participants who underwent health checkups from January to February 2023
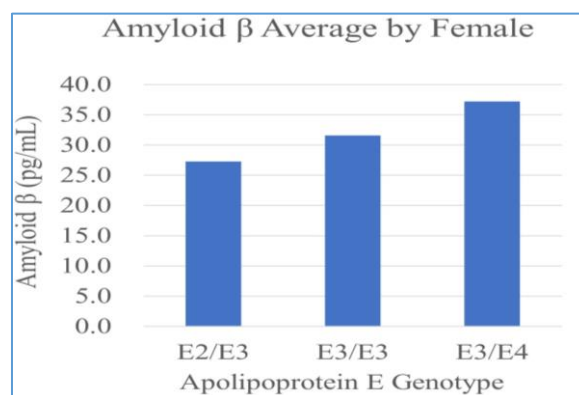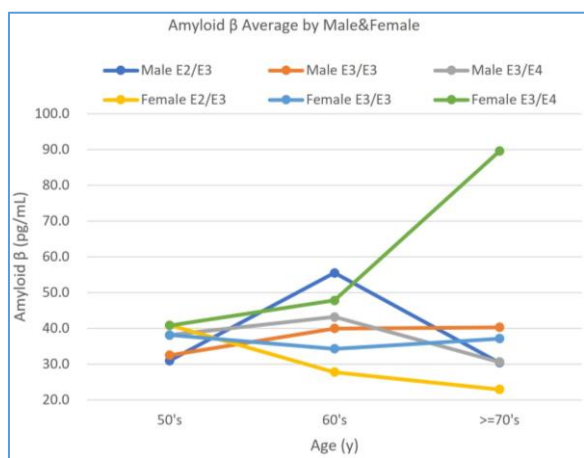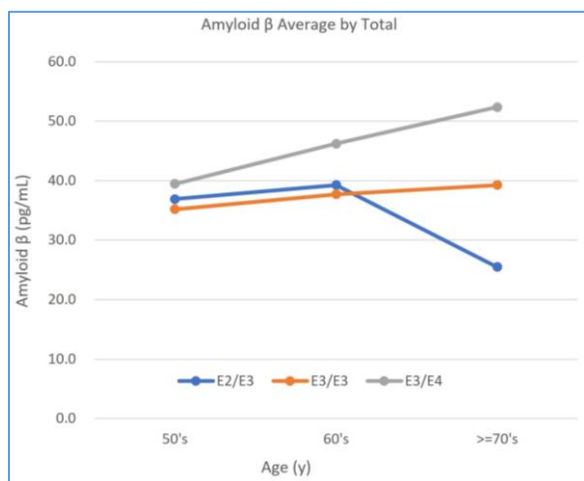

Fig. 2: Apolipoprotein E genotype of and amyloid β distribution in female participants who underwent health checkups from January to February 2023

Table 1: Distribution of apolipoprotein E genotypes in the general population of individuals over 50 years of age who underwent health checkups

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Apolipoprotein E genotype	Total (n)	Male individuals	Female individuals	Male individuals	Female individuals
E2/E2	3 (0.70)	2 (66.7)	1 (33.30)	—	—
E2/E3	60 (13.1)	28 (46.7)	32 (53.30)	0.945	<0.05*
E3/E3	308 (67.1)	175 (56.8)	133 (43.20)	0.220	0.536
E2/E4	8 (1.70)	3 (37.5)	5 (62.50)	—	—
E3/E4	78 (17.0)	43 (55.1)	35 (44.90)	0.708	0.106
E4/E4	2 (0.40)	0 (0.00)	2 (100.0)	—	—
Total	459	251 (54.7)	208 (45.30)		

Data are presented as n (%); *p<0.05

Fig. 3: Total, male and female apolipoprotein E genotypes and amyloid β distribution according to the sex and age of participants (50-70 years and older) who underwent health examinations from January to February 2023

Age Groups 40-49 and ≥70 Years

In the ≥70-year group, the mean plasma Aβ level in E3/E4 ApoE genotype individuals was the highest at 52.4 pg/mL and in E2/E3 ApoE genotype individuals, the Aβ level was the lowest at 25.5 pg/mL (Fig. 3).

The mean plasma Aβ level in male participants with E2/E3 ApoE genotypes aged 50-59 years was the highest at 55.5 pg/mL (Fig. 3). The mean plasma Aβ level in male participants with E2/E3 ApoE genotypes aged 40-49 years was the lowest at 31.0 pg/mL. The mean plasma Aβ level in female participants with E3/E4 ApoE genotypes aged ≥70 years was the highest at 89.6 pg/mL. The mean plasma Aβ level in female participants with E2/E3 genotypes aged ≥70 years was the lowest at 22.9 pg/mL (Fig. 3). Female participants with the E3/E4 genotype aged ≥70 years showed the highest Aβ levels and the Aβ levels were the highest in male participants with the E2/E3 genotype aged <50-59 years. In the analysis using the post-hoc test, there was no significant difference in E2/E3 ApoE genotypes (0.945), E3/E3 (0.220), E3/E4 (0.708) and age in male participants (Table 1). In the analysis using the post-hoc test, the ApoE genotype E2/E3 showed a significant difference with age in female participants (p<0.05) and E3/E3 and E3/E4 showed no significant difference with age (Table 1).

Figure 3 Total, male and female apolipoprotein E genotypes and amyloid β distribution according to the sex and age of participants (50-70 years and older) who underwent health examinations from January to June 2023.

Discussion

The E4 allele is a major genetic risk factor for late-onset AD. It increases the risk of developing AD by two- to four-fold (De Rojas *et al*., 2023; Neu *et al*., 2017), considerably affecting treatment outcomes (Nakamura *et al*., 2018). Allele E3 is the most common, followed by E4 and E2; however, their frequencies vary among populations (Liu *et al*., 2013; 2014). Among the alleles identified in our study, E3 was the most common and its occurrence varied with age.

ApoE4-related functional brain abnormalities, identified as reduced cerebral glucose metabolism in AD-related brain regions, have also been observed in healthy volunteers aged 20-39 years. In our study, E4 was the most common allele in participants younger than 30 years.

Here, the Aβ level in female participants increased with age in the presence the E4 ApoE genotype. Sex differences in age-related disorders are often linked to the

primary sex steroid hormone in women, the estrogen 17b-estradiol (Pike, 2017). Consistent with this possibility, some studies suggest that a decrease in estrogen levels in adulthood is associated with an increased risk of AD in women (Pike, 2017), indicating a positive correlation between low estrogen levels and AD.

AD is characterized by the abnormal deposition of Aβ in neurons and formation of extracellular plaques responsible for the pathologic events, causing neuronal degeneration and synapsis dysfunction (Bianchi, 2022). Aβ deposition and the Aβ protein precursor are mostly regulated by Testosterone (T) pathways (Bianchi, 2022). Therefore, sex hormones are relevant in AD development, as evidenced by the greater incidence of AD in women than in men (Bianchi, 2022).

Studies using cellular and animal models of AD have demonstrated that the T level is closely associated with neuronal efficiency and reduced Aβ deposition in the brain (Bianchi, 2022). The pathogenesis of AD in men has been attributed to low serum T levels (Bianchi, 2022).

Another study demonstrated that a low serum T level in men is associated with increased Aβ deposition, causing AD development and synaptic dysfunction with a consequent cognitive decline (Bianchi, 2022). In the present study, the Aβ level in male participants decreased with age in the presence of ApoE4. The sex-dependent role of ApoE4 in the risk of developing mild cognitive impairment (MCI) and the conversion from MCI to AD has recently been investigated, with evidence that women are at a greater risk than men (Altmann *et al.*, 2014; Ungar *et al.*, 2014). Studies on animals and humans have reported an interaction among ApoE4, menopause and cognitive decline (Riedel *et al.*, 2016). However, in our study, the frequency of the E4 allele was higher in male participants than in female participants. This difference can be attributed to the fact that the ApoE genotype analysis was performed on individuals undergoing health checkups, not those from hospitals, retirement homes and aging consortiums.

This study has some limitations. First, considerable biotemporal variability within individuals can be caused by various factors, including environmental stressors, sleep, age, diet and disease (Mielke *et al.*, 2014). We focused on the analysis of ApoE4 genotype and human Aβ level as predictors of AD development. The study participants were healthy individuals and information on other genetic markers associated with AD was not considered. Therefore, further well-designed studies are needed to understand the genetic and environmental risk factors of ApoE and human Aβ. Second, although ApoE polymorphism is a genetic risk factor for AD development, The majority of people in some regions do not carry this genotype. However, because our study involved a small number of participants, errors may have occurred during the analysis of the ApoE genotypes. Therefore, further studies with a higher number of patients of a wide age range are needed.

Conclusion

Our study provides age-based ApoE genotyping and Aβ analysis data from healthy Korean adults, highlighting the importance of ApoE4 and Aβ as predictors of AD risk and identifying age-specific differences. Although there is no cure for AD, identifying individuals who are genetically at a high risk may promote early clinical diagnosis and improve prognosis. Nevertheless, further research is needed to fully characterize the neuroinflammation-related changes in AD and determine their usefulness as biomarkers for AD diagnosis.

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Funding Information

Author's Contributions

Eun Ju Oh and Jung Jae Lee: Made substantial contributions to the conception and design of the study. These authors contributed equally to this study.

Bo Kyeung Jung: Made substantial contributions to the acquisition and analysis of data.

Jae Kyung Kim: Made substantial contributions to the acquisition and analysis of data and design of the study.

Ethics

This study was approved by the Institutional Review Board of Dankook University, Republic of Korea (No. 2023-10-020) and was conducted following the principles of the Declaration of Helsinki. The requirement for participant consent was waived because the statistical data of tests conducted by medical institutions for diagnosis did not include any personal information of the participants.

Conflict of Interest Disclosure

The authors declare that they have no competing interests.

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