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The sex-specific effect of the apolipoprotein E allele and methylenetetrahydrofolate reductase gene polymorphism on the biochemical, anatomical, and cognitive profiles of patients clinically diagnosed with probable Alzheimer's disease

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ABSTRACT

Objectives: We aimed to evaluate the sex-specific effect of apolipoprotein E (*APOE*) alleles and methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism on the biochemical, anatomical, and cognitive profiles of Alzheimer's disease (AD) patients.

Methods: The patient (followed-up for at least 2 years) medical records, which comprised of data on plasma homocysteine and folate levels, lipid profile, HbA1c, *MTHFR* C677T genotype, *APOE* allele type, mini-mental state examination (MMSE) and clinical dementia rating (CDR) scores, and brain scans, were retrospectively analyzed. Two trained neurologists scored the white matter lesions (Fazekas scale), medial temporal lobe atrophy (MTA), and microbleeds using brain magnetic resonance imaging scans.

Results: This study included 574 patients clinically diagnosed with probable AD (average age, 73.2 years; mean MMSE score, 10.05). The effect of sex on all parameters was evaluated. The triglyceride (TG) and homocysteine levels and the MTA and Fazekas scores were higher in female *APOE-ε4/ε4* carriers than in women without *APOE-ε4*. The TG and homocysteine levels were lower in men with the *MTHFR* CC allele than in those with the *MTHFR* TT allele. In contrast, *MTHFR* polymorphism and *APOE-ε4* alleles were not significantly correlated with anatomical lesions and rate of decline in the MMSE and CDR scores.

Conclusions: We demonstrated the sex-specific effect of the *APOE* allele and *MTHFR* polymorphism on the serological and anatomical biomarkers in AD patients. The *APOE* allele and *MTHFR* mutations did not directly affect cognitive progression, but differentially affected other biochemical factors, between the sexes. These findings will aid in devising novel preventive and therapeutic strategies.

Hee-Jin Kim and Junhui Park contributed equally.

KEYWORDS
Alzheimer's disease, *APOE* genotype, cognition, *MTHFR* C677T polymorphism, sex-specific effect

1 | INTRODUCTION

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases. According to World Health Organization, around 50 million people are suffering from dementia. Varying incidence and prevalence ranges of AD have been presented in several epidemiological studies. In the American and European AD cohorts, incidence and prevalence were 1.6–8.6 per 1000 person-years and 8.6%, respectively.[1] In a European meta-analysis study, AD incidence and prevalence were 11.08 per 1000 person-years and 3.31%, respectively.[2] In a Korean study performed through a nationwide survey, AD incidence and prevalence were 7.9 per 1000-person years and 8.1%, respectively.[3] The factors that contribute to cognitive decline in AD patients include age, sex, and cardiovascular risk factors.[4] Old age and female sex are predominant risk factors of AD. AD prevalence and severity are high among women.[5] However, the biological mechanisms underlying the differential pathogenesis of AD between the sexes have not been understood well.[5] There is increased interest in analyzing the sex-specific differences in etiological factors, manifestations, treatment responses, and outcomes of neurological diseases.[6] Sex is determined based on chromosomes, hormones, or reproductive structures.[7] Genetic, biochemical, and pathological studies have suggested that amyloid beta (Aβ) aggregation is involved in initiating AD pathogenesis. Various genetic factors, including apolipoprotein E (*APOE*), contribute to clinical AD progression.[8] *APOE-ε4*, which is associated with increased risk of AD, is involved in various functions of the central nervous system (CNS), such as cholesterol transport, neuroplasticity, and inflammation.[9] Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The *MTHFR* c.677C > T (A222V) variant exhibits decreased MTHFR activity.[10] The T allele of the *MTHFR* c.677C > T gene is associated with folate and vitamin B12 deficiency and hyperhomocysteinemia (H-Hcy), which are conditions that impair methylation reactions. Additionally, *MTHFR* polymorphism, presenilin, and amyloid precursor proteins contribute to the increased risk of oligodendrocyte impairment.[11] Here, we examined the sex-specific effects of the *APOE-ε4* allele and *MTHFR* c.677C > T polymorphism on the biochemical, anatomical, and cognitive progression profiles of patients clinically diagnosed with probable AD. We examined the correlation of the *APOE-ε4* allele and *MTHFR* c.677C > T polymorphism with serological markers (homocysteine, folate, HbA1c, and lipid profile) based on the sex, cognitive progression over 8 years, magnetic resonance imaging (MRI) markers, and AD progression.

Key points

- This Asian population-based long-term study examined the clinical implication and sex-specific effect of apolipoprotein E (*APOE*) alleles and methylenetetrahydrofolate reductase (*MTHFR*) c.677C > T polymorphism on various biomarkers in Alzheimer's disease (AD) patients
- The number of *APOE-ε4* alleles was significantly correlated with enhanced homocysteine levels in female subjects and decreased homocysteine levels in male subjects, and the *APOE-ε4* carrier status was significantly correlated with medial temporal lobe atrophy (MTA), frontal atrophy, and periventricular and deep white matter lesions
- *MTHFR* polymorphism was associated with old age, high clinical dementia rating score, low folate level, and high HbA1c in males and aggravated anatomical biomarkers (MTA, Fazekas score, and number of microbleeds)
- Sex-specific genotypes might directly or indirectly affect the progression of AD and dementia

2 | MATERIALS AND METHODS

2.1 | Subjects

We performed a retrospective analysis of the medical records of 2414 patients diagnosed with clinical AD between 2007 and 2014 and followed-up for at least 2 years. Patients were diagnosed with AD based on the criteria for the diagnosis of clinical probable dementia due to AD: recommendations from the National Institute on Aging-Alzheimer's Association (NIA-AA) workgroups on the diagnostic guidelines of AD.[12] Patients who meet any of the following criteria related to CNS disorders were excluded: any evidence of a condition (other than AD) that may affect cognition, including frontotemporal dementia, dementia with Lewy bodies, vascular dementia, Parkinson's disease, corticobasal syndrome, Creutzfeldt–Jakob disease, progressive supranuclear palsy, frontotemporal lobar degeneration, Huntington disease, normal pressure hydrocephalus, seizure disorder, delirium, and hypoxia, and/or history or presence of clinically evident systemic vascular disease that in the opinion of the investigator has the potential to affect cognitive function. Patients who showed a history or the presence of clinically evident cerebrovascular disease and asymptomatic

developmental venous anomalies might be considered eligible after discussion. History or presence of any stroke with clinical symptoms within the past 12 months and documented history within the last 6 months of an acute event that is consistent with a transient ischemic attack were also considered exclusion criteria. Patients with a history of severe clinically significant CNS trauma or the presence of intracranial mass that could potentially impair cognition were excluded. Dementia in patients was evaluated based on the Korean version of the mini-mental state examination (MMSE)[13] and clinical dementia rating (CDR) scale.[14] The change in the MMSE score each year (ΔMMSE) was calculated as follows: (current MMSE score − previous MMSE score)/number of years between the current and previous MMSE scores. Information on medical history, present illness, and family history of dementia was obtained from the participants and their family members. Blood samples were collected to measure folate, vitamin B12, and homocysteine levels. *MTHFR* c.677C > T polymorphism and *APOE-ε4* allele were identified using genotype analysis. Additionally, the demographic data, including age, sex, and education, were collected. All participants provided their informed consent to participate in the study. This study was approved by the Hanyang University Hospital Institutional Review Board (HYUH 2017-08-034-004).

2.2 | Serum and genotypic analyses

When possible, fasting blood samples were collected in the morning. The samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged to separate the plasma, and stored at −70°C within 2 h of collection. The serum folate and vitamin B12 levels were determined by an immunoassay. The total plasma homocysteine level was measured by high-performance liquid chromatography. *MTHFR* c.677C > T gene polymorphism was identified by polymerase chain reaction after *Hin*FI restriction digestion, as described previously with minor modifications.[10] Allele frequencies were estimated based on gene counting. The observed numbers of each genotype were compared with those expected under the Hardy–Weinberg equilibrium. *APOE* genotyping was performed as described previously with minor modifications.[15] *APOE* genotype was classified according to the variations of the *APOE-ε* allele (2/2, 2/3, 3/3, 2/4, 3/4, and 4/4).

2.3 | Visual rating of anatomical biomarkers

Anatomical biomarkers were evaluated based on visual rating scales. Conventional MRI was performed using a 3.0 T MRI scanner (Philips Real Time Compact Magnet 3.0-Tesla MRI system, Achieva 3.0-Tesla X-series) with an 8-element phased-array neurovascular array coil. All subjects underwent magnetic resonance scanning following a standard protocol. The scanning series were as follows: axial T2-weighted images (echo time [TE], 100–120 ms; repetition time [TR], 4000–6000 ms; voxel size, 1 × 1 × 5–7.5 mm^3; 19–24 slices), axial fluid-attenuated inversion recovery (FLAIR) images (TE, 100–140 ms; TR, 6000–10 000 ms; inversion time, 2000–2400 ms; voxel size, 1 × 1 × 5–7.5 mm^3; 19–24 slices), and a volumetric three-dimensional spoiled fast gradient echo (TR, 7.3 ms; TE, 2.7 ms; slice thickness, 1.0 mm; flip angle, 13°; and field of view, 256 × 256 mm [based on T1-weighted images]). The white matter lesions in the FLAIR images were scored based on the Fazekas visual rating scales (0–3).[16] Medial temporal lobe atrophy (MTA) in the T1 coronal images was scored based on the Scheltens scale (0–4).[17] Frontal atrophy (FA) in the axial T1-weighted images was scored based on the global cortical atrophy scale, following the modified criteria described by Pasquier et al.[18,19] The number of microbleeds was analyzed in the susceptibility-weighted images following the recently published guidelines.[20,21] All ratings were calculated by two experienced neurologists who were blinded to the clinical data.

2.4 | Statistical analyses

The clinical data of the participants were analyzed using descriptive statistics and Student's t-test. The means were compared by analysis of variance. All data are presented as the mean ± standard deviation. The Hardy–Weinberg equilibrium of the *MTHFR* genotypes was examined using the Chi-squared test. The correlation between the *MTHFR* genotype and other variables was assessed using Pearson's correlation analysis. The effect of the *MTHFR* genotype on baseline clinical and biochemical variables was examined using analysis of co-variance (ANCOVA). The correlation between the genotypes and homocysteine concentrations was determined after adjusting the data for age, sex, and plasma folate concentrations (as a continuous variable within plasma folate categories). The sex-specific effects of the *APOE* allele and *MTHFR* genotype on the serological, anatomical, and cognitive progression markers were examined. Proportional odds model was used to evaluate the correlation of the *APOE* allele with MTA and white matter lesions. The Jonckheere-Terpstra test was used to determine the significant correlations of the *MTHFR* and *APOE* genotypes with baseline clinical and biochemical variables. Multiple logistic regression analysis was used to evaluate the factors that affected ΔMMSE. Patients who exhibited a change in ΔMMSE were divided into the following two groups based on the median zero using odds ratios (OR): decliners, patients experiencing negative change; nondecliners, patients experiencing positive change. Differences were considered statistically significant at p-value <0.05. All statistical analyses were performed using SPSS version 24.0 (SPSS Inc.) and SAS version 9.4 (SAS Institute Inc.). A logistic regression model was applied to adjust the data for age; triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol levels; and HbA1c.

3 | RESULTS

3.1 | Patient characteristics

The medical records of 2414 dementia patients who underwent genetic, serological, neuropsychological, and structural neuroimaging

tests were retrospectively analyzed (Figure S1). In total, 574 patients (413 women and 161 men) clinically diagnosed with probable AD based on the NIA-AA criteria were included. Patient characteristics based on CDR scores are shown in Table 1. Patients with CDR ≥2.0 were associated with advanced age, low MMSE score, and increased homocysteine levels (CDR ≤0.5, 12.1 ± 6.3; CDR = 1.0, 13.2 ± 7.0; CDR ≥2.0, 17.0 ± 12.2; $p < 0.001$). The visual rating scores of the brain MRI scans revealed differences in the gray and white matter lesions between the groups. Patients with CDR ≥2.0 exhibited high MTA score and extensive lesions in the deep and periventricular white matter. The prevalence of microbleeds was higher in patients with CDR ≥2.0 (0.46 ± 0.71) than in those with CDR ≤0.5 (0.21 ± 0.55) or CDR = 1.0 (0.31 ± 0.64; $p < 0.001$).

3.2 | Serological and anatomical biomarkers

Serum total cholesterol, HDL-cholesterol, vitamin B12, and folate levels; *APOE* allele number; and *MTHFR* c.677C > T polymorphism were not significantly different between the groups. Age was significantly correlated with low education, high CDR score, MTA, and severe white matter lesions. Male subjects exhibited a significantly higher serum homocysteine level (15.8 ± 10.9 μmol/L vs. 12.4 ± 8.0 μmol/L, $p < 0.001$) and a lower folate level (8.6 ± 4.01 mg/dl vs. 9.7 ± 4.1 mg/dl, $p < 0.001$) than those of female subjects. The HDL- and total cholesterol levels were significantly higher in female subjects than in male subjects. Total cholesterol was correlated with other lipid profiles (HDL- and LDL-cholesterol and TG). Enhanced serum TG level was correlated with enhanced total cholesterol and low HDL-cholesterol levels. The serum total cholesterol level was inversely correlated with the number of microbleeds in the brain. Increased HbA1c levels were correlated with high TG levels ($r = 0.16$, $p = 0.005$) and high MTA scores ($r = 0.12$, $p = 0.03$). H-Hcy was correlated with advanced age, high CDR, low folate level, and aggravated anatomical biomarkers (MTA, Fazekas score, and number of microbleeds; Tables S1 and S2). Low folate level was correlated with high MTA score. HbA1c was correlated with high TG levels, H-Hcy, and high MTA score. The severity of anatomical biomarkers was correlated with female sex, old age, low education, and high CDR. MTA was significantly correlated with female sex, old age, low education, high CDR, H-Hcy, low folate, and other anatomical biomarkers.

3.3 | Correlation of *MTHFR* c.677C > T polymorphism with serological and anatomical biomarkers

Serum homocysteine levels were significantly higher in patients with the *MTHFR* TT allele than in those with the *MTHFR* CC allele ($p = 0.0051$; Table S3, Figure 1A). Enhanced homocysteine levels were associated with male subjects, especially old male subjects with the *MTHFR* TT allele. Folate levels did not significantly vary between patients with the *MTHFR* TT allele and those with other alleles. No correlation between *MTHFR* polymorphism and other serological biomarkers (TG, LDL-cholesterol, HDL-cholesterol, and HbA1c) was observed. However, patients with the *MTHFR* TT allele, especially, male and old age subjects, exhibited hypertriglyceridemia and enhanced homocysteine levels (Table 2). *MTHFR* polymorphism was not significantly correlated with anatomical biomarkers (MTA, FA, white matter lesions, or number of microbleeds). The *MTHFR* TT genotype was not significantly associated with the MMSE and CDR score decline rates.

3.4 | The sex-specific effect of the *APOE-ε4* allele on the serological and anatomical biomarkers

The *APOE-ε4* carrier status was positively correlated with the LDL- and total cholesterol levels ($r = 0.22$, $p < 0.0001$ vs. $r = 0.21$, $p < 0.0001$) after adjustment for the *MTHFR* genotype (Figure 1B, Table S1). H-Hcy was significantly associated with female *APOE-ε4* carriers ($p = 0.0184$). *APOE-ε4* was not significantly correlated with H-Hcy when the sex was not considered. The number of *APOE-ε4* alleles was significantly correlated with enhanced homocysteine levels in female subjects and decreased homocysteine levels in male subjects (Table 2). The *APOE-ε4* carrier status was significantly correlated with MTA, FA, and periventricular and deep white matter lesions. The presence of two *APOE-ε4* alleles (OR = 2.351), age (OR = 0.1265 or 1.135), and female sex (OR = 2.095) were associated with increased MTA. The number of *APOE-ε4* alleles was significantly associated with FA (OR = 1.2440, $p = 0.0045$). The *APOE-ε4* carrier status was associated with increased periventricular (OR = 1.45, $p = 0.03$) and deep white matter lesions (OR = 1.01, $p = 0.005$).

3.5 | Predictive factors of the MMSE score decline rate in subjects clinically diagnosed with probable AD

In the multiple logistic regression analysis, ΔMMSE was considered a dependent variable (with age, sex, total cholesterol, TG, LDL-cholesterol, HDL-cholesterol, serum homocysteine, folate, genotype, and visual rating scales for anatomical biomarkers as the individual variables) to determine the factors that affected ΔMMSE. The LDL- and total cholesterol levels, *APOE* genotype, and periventricular white matter lesions were the predictive factors of ΔMMSE score decline. Age was associated, but not significantly, with the MMSE score decline. *MTHFR* c.677C > T polymorphism was not significantly different between the decliner and nondecliner groups. However, patients with two *APOE-ε4* alleles exhibited lower ΔMMSE scores (OR = 14.01 [1.05–185.9], $p = 0.045$) than those with one *APOE-ε4* allele (OR = 7.91 [1.34–46.6], $p = 0.022$.) The LDL- (OR = 0.98 [0.97–0.99], $p = 0.029$) and total (OR = 1.03 [1.00–1.02], $p = 0.022$) cholesterol levels and periventricular white matter lesions (OR = 1.52 [1.14–2.04], $p = 0.004$) affected ΔMMSE. These results are demonstrated in Table 3.

TABLE 1 Demographic and descriptive data of the study subjects

	CDR ≤0.5, n = 227	CDR = 1.0, *n* = 236	CDR ≥2.0, *n* = 111	*p*-value
Age, mean (SD)	68.0 (10.3)	74.4 (8.0)	80.9 (8.3)	<0.001*
Range (years)	48–90	45–95	59–91	
Number of women (%)	163 (71.9)	169 (71.6)	81 (71.9)	0.96
MMSE, mean (SD)	23.7 (3.2)	18.5 (3.1)	13.4 (4.5)	<0.001*
Range	22–29	14–25	7–19	
△MMSE/△year, mean (SD)	0.18 (1.83)	0.32 (1.81)	0.17 (2.48)	0.85
Range	−10–9.5	−6–9	−5–3	
CDR, mean (SD)	0.4 (0.18)	1.0 (0.00)	2.17 (0.5)	0.006*
HbA1c, mean (SD)	6.07 (1.06)	6.12 (1.20)	6.27 (1.17)	0.42
Range (%)	4.8–11.6	4.4–14.4	4.6–5.9	
Cholesterol, mean (SD)	201.5 (36.2)	195.5 (39.8)	196.0 (44.9)	0.58
Range (mg/dl)	141.4–303.0	115.0–312.0	97.0–250.2	
TG, mean (SD)	135.3 (76.2)	126.7 (67.4)	119.5 (56.0)	0.32
Range (mg/dl)	37–511	35–485	28–99	
HDL, mean (SD)	48.5 (12.7)	48.3 (12.7)	48.3 (14.8)	0.32
Range (mg/dl)	28–98	24–115	2–70	
LDL, mean (SD)	118.8 (36.2)	114.7 (32.5)	113.4 (38.2)	0.47
Range (mg/dl)	72–352	52–214	35–166	
Vitamin B_{12}, mean (SD)	660.3 (273.5)	687.8 (381.5)	635.4 (411.1)	0.18
Range (pmol/L)	521.4–925.0	412.0–946.0	113.9–2000.0	
Folate, mean (SD)	9.74 (3.73)	8.97 (4.15)	8.62 (4.62)	0.22
Range (nmol/L)	4.0–20.0	3.0–28.0	2.0–20.0	
Homocysteine, mean (SD)	12.1 (6.3)	13.2 (7.0)	17.0 (12.2)	<0.001*
Range (μmol/L)	6–59	4–95	6–18	
APOE allele				0.582
2/3 (%)	5.7	6.1	3.8	
3/3 (%)	55.2	56.5	68.4	
3/4 (%)	33.3	30.4	19	
4/4 (%)	4.2	3.9	5.1	
2/4 (%)	1	1.7	1.3	
MTHFR (C677T)				0.942
CC (%)	37	35.9	26.6	
CT (%)	42.2	48.3	59.5	
TT (%)	19.8	17.4	12.7	
MTA	1.47 ± 1.2	2.10 ± 1.1	2.77 ± 1.03	<0.001*
FA, mean (SD)	1.47 (0.79)	1.74 (0.75)	2.26 (0.74)	<0.001*
Number of microbleeds, mean (SD)	0.21 (0.55)	0.31 (0.64)	0.46 (0.71)	0.008*

TABLE 1 (Continued)

	CDR ≤0.5, n = 227	CDR = 1.0, *n* = 236	CDR ≥2.0, *n* = 111	*p*-value
White matter lesions (D), mean (SD)	1.14 (0.88)	1.43 (0.94)	1.97 (0.86)	<0.001*
White matter lesions (P), mean (SD)	1.19 (0.87)	1.54 (1.02)	2.17 (0.90)	<0.001*

Abbreviations: *APOE*, apolipoprotein E; CDR, clinical dementia rating score; D, deep; FA, frontal lobe atrophy scored based on Pasquier's criteria; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MMSE: mini-mental status examination; MTA, medial temporal atrophy score based on Scheltens scale; *MTHFR*, methylenetetrahydrofolate reductase; P, periventricular; SD, standard deviation; TG, triglyceride.

*Statistically significant at $p < 0.05$.

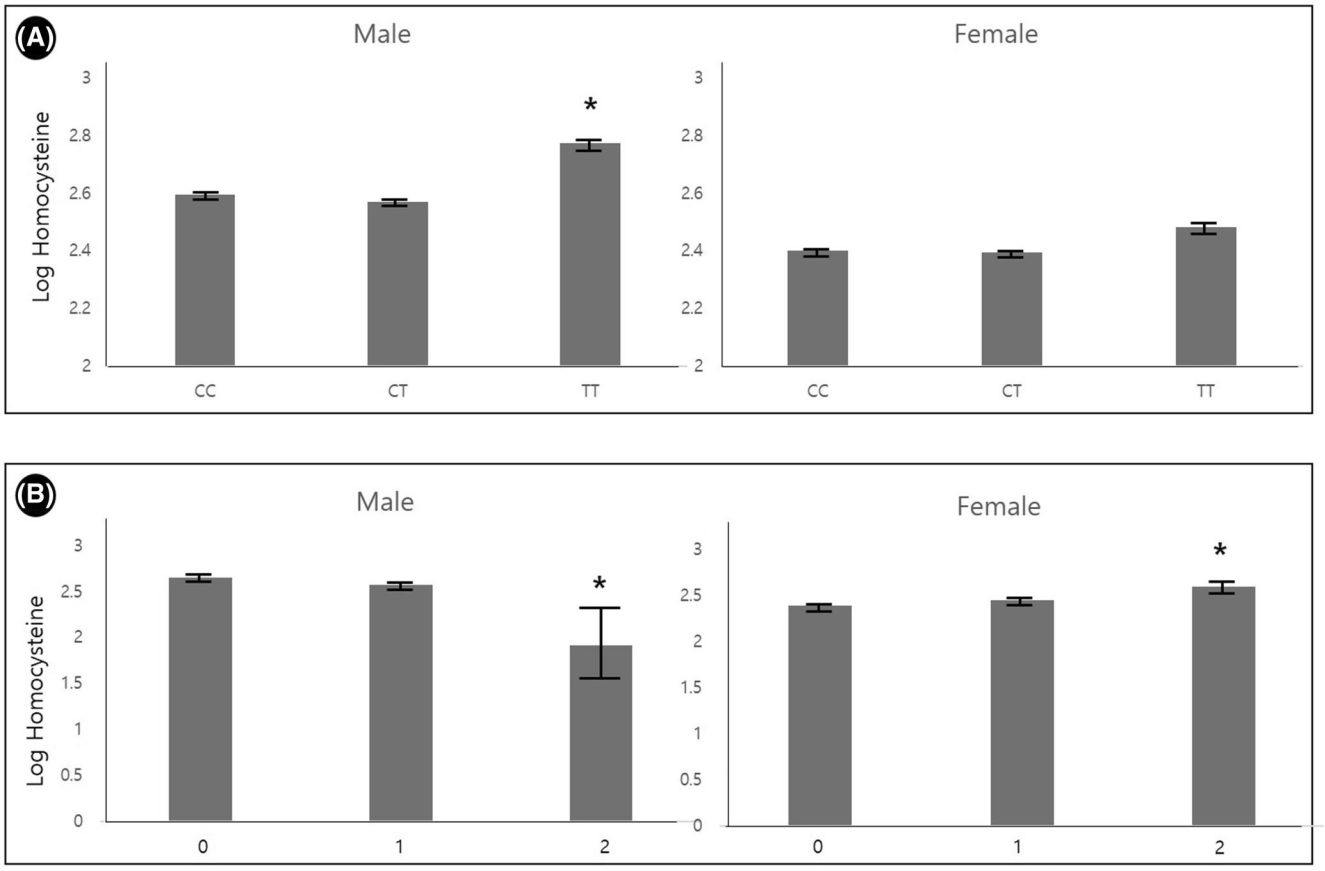

FIGURE 1 (A) *MTHFR* polymorphism and homocysteine showed trends toward higher homocysteine levels going from the CC allele to the TT allele (*p*-value = 0.0053) by ANCOVA with a linear contrast. Error bar, standard error; sex, *p*-value <0.0001. (B) *APOE-ε4* (APOE_e4) and homocysteine showed trends toward higher homocysteine levels going from the non-*APOE-ε4* carrier status to *APOE-ε4/ε4* in females and the opposite trend in males. Error bar, standard error; ApoE*sex, *p*-value = 0.0184. 0, 1, and 2, numbers of *APOE-ε4*; ANCOVA, analysis of co-variance; *APOE*, apolipoprotein E; *MTHFR*, methylenetetrahydrofolate reductase

4 | DISCUSSION

AD is characterized by extensive neuronal loss and accumulation of intracellular neurofibrillary tangles and extracellular amyloid plaques in the brain.[22] In this Asian population-based study, the sex-specific effect of genetic polymorphism on various biomarkers in AD patients was examined. We hypothesized that the *APOE* allele type and *MTHFR* c.677C > T mutation directly or indirectly affected the biochemical, clinical, and anatomical risk factors of AD and consequently the MMSE scores. Additionally, we assumed that the correlation between the genes and other factors was sex-specific. Previously, we have demonstrated that metabolic risk factors vary between the sexes. The combination of metabolic risk factors and advanced age, low education levels, history of stroke, lack of exercise, and hypertension was associated with increased risk of dementia in men. Advanced age, low education levels, history of stroke, lack of exercise, hypertension,

TABLE 2 The different effects of *MTHFR* c.677C > T polymorphism and *APOE-ε4* on other serological markers

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	*p*-value
MTHFR T allele	2	1.29139734	0.6456987	5.91	0.0029
Sex	1	4.52471892	4.5247189	41.38	<0.0001
T allele*sex	2	0.2073508	0.1036754	0.95	0.3881
Age	1	15.96649802	15.966498	146.02	<0.0001
APOE-ε4	2	0.263785	0.131893	1	0.368
Sex	1	0.075077	0.075077	0.57	0.4506
ε4*sex	2	1.059821	0.52991	4.02	0.0184
Age	1	17.76239	17.76239	134.87	<0.0001

Note: The values for different *MTHFR* genotypes (mean ± standard deviation) were analyzed using analysis of variance. The genotype distribution did not significantly deviate from the Hardy–Weinberg equilibrium.

Abbreviations: *APOE*, apolipoprotein E; *MTHFR*, methylenetetrahydrofolate reductase.

TABLE 3 Predictive variables of the rate of decline in the MMSE score of patients clinically diagnosed with probable AD

Factor	OR	P	95% CI	SE	Wald
Age	1.027	0.093	0.996–1.059	0.016	2.822
Cholesterol	1.013*	**0.022**	1.002–1.024	0.006	5.239
LDL	0.986*	**0.029**	0.973–0.999	0.007	4.783
APOE allele		0.056			9.233
APOE 2/3	2.553	0.173	0.663–9.829	0.688	1.856
APOE 3/3	4.045*	**0.049**	1.004–16.3	0.711	3.861
APOE ¾	7.919*	**0.022**	1.345–46.611	0.904	5.235
APOE 4/4	14.01*	**0.045**	1.055–185.997	1.319	4.003
White matter lesion (P)	1.528*	**0.004**	1.145–2.039	0.147	8.297

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CI, confidence interval; LDL, low-density lipoprotein cholesterol; MMSE, mini-mental state examination; OR, odds ratio; P, periventricular; SE, standard error; Wald, Wald χ2.

*Statistically significant at $p < 0.05$.

diabetes mellitus, and alcohol abstinence increased the prevalence of dementia among women in a community-based study.[23]

In this study, *MTHFR* polymorphism was strongly correlated with H-Hcy, especially among male subjects. The *APOE-ε4* allele was correlated with increased serum LDL- and total cholesterol levels, MTA, and deep white matter lesions. The *APOE-ε4* carrier status was not significantly correlated with H-Hcy when sex was not considered. However, the *APOE-ε4* carrier status in females was significantly correlated with H-Hcy ($p = 0.0184$). Homocysteine levels were directly and inversely correlated with the number of *APOE-ε4* alleles in males. This indicated that the effect of *APOE-ε4* on homocysteine levels was female sex-specific. Age was significantly correlated with *APOE-ε4*. However, the sex-specific effect of the *APOE-ε4* allele and *MTHFR* gene polymorphism on progressive cognitive decline in AD patients was not significant.

Patients with the *MTHFR* TT allele showed H-Hcy and decreased folate levels. H-Hcy was correlated with male sex, advanced age, high CDR, low folate level, high HbA1c, and aggravated anatomical biomarkers (MTA, Fazekas score, and number of microbleeds). Enhanced plasma homocysteine level is an independent risk factor of AD.[6,24] H-Hcy is also associated with MTA[25] and is an independent risk factor of moderate to severe leukoaraiosis in individuals with AD and deep white matter lesions.[26] Advanced age; male sex; renal dysfunction; genetic variants; high uptake of methionine from protein-rich food; and folate, vitamin B12, and vitamin B6 deficiencies are associated with H-Hcy.[27] Homocysteine, a toxic intermediate of methionine metabolism, is irreversibly metabolized through the vitamin B6-dependent *trans*-sulfuration pathway. Alternatively, homocysteine is recycled into methionine via the folate- and vitamin B12-dependent remethylation pathway.[28] Studies on cell cultures revealed that the potential mechanisms underlying the correlation between enhanced homocysteine levels and AD include induction of vascular changes and cortical neuron excitotoxicity, indicating that homocysteine is the causative factor of cholinergic deficiency in AD.[29] Dysregulated folate metabolism is correlated with enhanced homocysteine levels, which exert neurotoxic effects via oxidative damage and excitotoxicity.[30] In this study, patients with the *MTHFR* TT genotype and advanced AD exhibited H-Hcy and low folate levels.

The correlation between the *MTHFR* T allele and H-Hcy was stronger among men than among women. *MTHFR* mutation is associated with increased mortality among middle- and old-aged men, but not women.[31] The effect of the *MTHFR* C677T genotype on plasma homocysteine concentrations in healthy children is sex-specific.[32] Genetic factors also affect the homocysteine level, which is dependent on the gene-nutrient interactions. The *MTHFR* C677T variant, a total homocysteine metabolism-regulatory enzyme, is an important genetic determinant of total homocysteine levels.[32] The total homocysteine levels were higher in male subjects with the homozygous *MTHFR* TT allele than in those with the heterozygous CT or homozygous CC alleles. In contrast, the *MTHFR* genotype did not affect the total homocysteine levels in female subjects.[32] HbA1c was positively correlated to H-Hcy. H-Hcy has been reported frequently in type II diabetes patients who exhibit enhanced HbA1c levels.[33] Hyperglycemia and H-Hcy, which impair the microcirculation, are potential risk factors of atherosclerosis.[33] However, whether enhanced homocysteine level is a cause or consequence of dementia remains unclear. Recently, the prevalence of this mutation was found to vary depending on the ethnicity and geographical location.[34] *MTHFR* c.677C > T polymorphism is reported to be associated with AD in Asian populations, but not Caucasian populations.[34] In this study, no direct correlation between the *MTHFR* TT allele and other biomarkers (except for H-Hcy) was observed. This indicated that *MTHFR* c.677C > T polymorphism indirectly mediated AD pathogenesis by increasing the homocysteine level or through other biochemical pathways, including enhanced glucose and lipid metabolism, and promoted vascular injury, which may be sex-specific, in AD.

The *APOE-ε4* allele was correlated with female sex and positively correlated with LDL- and total cholesterol levels in patients clinically diagnosed with probable AD. The *APOE-ε4* allele carrier status was strongly associated with MTA and deep white matter lesions. The characteristic neuropathological hallmarks of AD include senile plaques, neurofibrillary tangles, and synapse loss.[35] The *APOE-ε4* allele, which is involved in Aβ aggregation and clearance, increases the risk of developing AD. APOE is the major cholesterol carrier in the brain.[36] APOE-mediated binding of Aβ to the cell surface may be isoform-specific (ε2 >ε3 >ε4).[37] APOE may also mediate Aβ internalization by binding to the LDL receptor-related protein.[36] The *APOE-ε4* allele is associated with hyperlipidemia and hypercholesterolemia, which lead to atherosclerosis, coronary heart disease, and stroke.[36] No significant correlation between H-Hcy and *APOE-ε4* was observed. However, the *APOE-ε4* allele carrier status in females exhibited significant correlation with H-Hcy, possibly explaining the correlation between *APOE-ε4* and increased deep white matter lesions. The white matter lesions that affected the MMSE score decline were observed in the periventricular area (OR = 1.528). The *APOE-ε4* allele is reported to be a vascular risk factor associated with neurovascular dysfunctions independent of other factors, such as hypertension, diabetes, or dyslipidemia.[36] The MMSE score decline rate was higher in patients with two *APOE-ε4* allele copies than in those with one *APOE-ε4* allele copy (OR = 14.01 vs. OR = 7.91) and those with the *APOE-ε3/ε3* genotype (OR = 4.04). A meta-analysis of clinical and autopsy-based studies demonstrated that the risk of AD was higher in individuals with one (ε2/ε4, OR 2.6; ε3/ε4, OR 3.2) or two (ε4/ε4, OR 14.9) *APOE-ε4* allele copies than in those with the *APOE-ε3/ε3* genotype.[38]

Furthermore, total cholesterol was correlated with other lipid profiles. Elevated TG levels were correlated with elevated total cholesterol and decreased HDL-cholesterol levels. LDL-cholesterol level was correlated with the *APOE* allele type, but not *MTHFR* polymorphism. High total cholesterol or low HDL-cholesterol level is a risk factor of AD.[39] Even moderately elevated cholesterol level in midlife increases the risk of dementia.[40] The Honolulu-Asia Aging Study (HAAS) reported no correlation between midlife serum total cholesterol level and risk of AD, even though a cluster of cardiovascular metabolic risk factors at midlife increased the risk of dementia.[41] In this study, the factors that affected the MMSE score decline rate were LDL- and total cholesterol, suggesting that the serum cholesterol level could predict the progression of symptoms and was a risk factor of AD. Interestingly, the blood total cholesterol concentration was inversely correlated with the number of microbleeds in the brain. This was consistent with the results of a previous study that reported the correlation between enhanced cortical microbleeds and old age, male sex, low total cholesterol level, *APOE* carrier status, and statin usage.[42] The correlation between cerebral microbleeds and dementia remains controversial.[21] Hence, further studies are needed to clarify this correlation in AD.

MTA severity is also related to H-Hcy and low serum folate levels. MTA severity was correlated with female sex, old age, low education, and high CDR score based on the *APOE* allele types, but not *MTHFR* polymorphism. Other anatomical biomarkers, such as deep and periventricular white matter lesions and FA, were correlated with MTA. Periventricular white matter lesions affected the ΔMMSE score decline rate, but not MTA. Patients with large white matter lesions in the frontal cognitive domains performed significantly worse than those with small lesions in the Framingham Heart Study.[43] The clinical importance of mixed pathology, including vascular lesions, has been constantly reported.[44]

This longitudinal (8 years) follow-up study has some limitations. This study was a single center study. The effects of *MTHFR* cannot be determined based on the MMSE score because MMSE is not a complex neuropsychological test. Although we introduced ΔMMSE to reflect the serial change in the patient cognitive status, this study was performed at the time of *MTHFR* sampling. The clinical significance of *MTHFR* polymorphism should be evaluated based on continuous serum results.

In conclusion, the direct effects of *MTHFR* on AD were not identified in this study. However, *MTHFR* mutations were associated with H-Hcy, which might indirectly affect AD progression. These findings may aid in devising preventive and treatment strategies for AD. H-Hcy was correlated with male sex, old age, high CDR, low folate level, high HbA1c, and aggravated anatomical biomarkers (MTA, Fazekas score, and number of microbleeds). The results of the correlation between the *APOE-ε4* allele and serological markers, including LDL- and total cholesterol, in this AD cohort concurred with

those of previous studies. This correlation might be sex-specific. The predictive factors of the MMSE score decline were total cholesterol, LDL-cholesterol, the *APOE* genotype, and periventricular white matter lesions. The potential association of epigenetic effects with *MTHFR* gene mutations may provide new therapeutic targets for AD. Further prospective research focusing on epigenetic modification is needed to improve the outcomes of AD patients.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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