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ApoE4 genotype and cognitive performance in Korean health examinees with subjective memory complaints: A retrospective cross-sectional study

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Abstract

Introduction: Subjective memory complaints are a natural phenomenon in the aging process. The apolipoprotein E epsilon 4 (ApoE4) allele is the strongest genetic risk factor for Alzheimer's disease. The aims of this study were to determine the distribution of the ApoE genotype and how the status of ApoE4 is associated with the cognitive function of Korean health examinees with subjective memory complaints.

Methods: A retrospective cross-sectional study was conducted with 1,107 health examinees with subjective memory complaints who underwent both medical and cognitive testing including ApoE genotyping at two health-promotion centers in two Korean cities between 2016 and 2017. ApoE was genotyped using the Seeplex ApoE ACE Genotyping (Seegene, Seoul, Korea). Cognitive function was assessed using the K-MMSE. All of the subjects were classified according to the ApoE4 status into non-carriers, heterozygotes, and homozygotes. The odds ratios (ORs) were estimated using logistic regression for assessing the relationship between the ApoE4 genotype and cognitive impairment. ANOVA was used to compare the cognitive domain scores according to the ApoE4 status.

Results: ApoE4 allele frequency was 10.6%. ApoE4 homozygosity was significantly associated with a lower K-MMSE score (≤23) (OR=4.651, 95% CI=1.287–16.812) compared to ApoE4 non-carriers, and this association remained after adjusting for age, sex, BMI, blood pressure, blood lipid, and glucose. The scores in the temporal orientation and recall domains were lower in ApoE4 heterozygotes than in ApoE4 non-carriers (P<0.05).

Discussion: ApoE4 homozygosity was associated with cognitive impairment in health examinees with subjective memory complaints and ApoE4 allele selectively affects to temporal orentation and recall domains.

Introduction

Alzheimer's disease (AD) is a neurodegenerative brain disorder and the main cause of dementia. The duration of disease progression can range from several years to decades [1]. There is a transitional period between normal aging and the clinical diagnosis of AD. Although subjective memory complaints are a natural phenomenon in the aging process, it may be in this transitional zone such as subjective cognitive decline (SCD). SCD is a possible predictor of future cognitive decline [2]. Although individuals with SCD would remain stable or revert to a normal state [3], undetermined genetic factors are partly responsible for SCD progressing to dementia [4].

Genetically, the apolipoprotein E epsilon 4 (ApoE4) allele is the strongest genetic risk factor for AD. The ApoE gene has three common alleles (E2, E3, and E4). ApoE4 carriers are at higher risks of both early-onset AD and late-onset AD compared with ApoE3 carriers [5,6]. With regard to nondemented people, there is controversy about the association between risk factors for cognitive impairment and ApoE4. Some studies [7-9] have observed ApoE4-related deficits in cognitive performance, while control studies [10,11] have found no difference in the prevalence of ApoE4 between healthy controls and SCD groups.

In addition, ApoE4 genotype distribution in not only the general population but also AD varies between different geographical regions [12]. For example, Norberg et al. [13] showed that the ApoE4 prevalence exhibits a north–south gradient across European both healthy people and AD, being highest in the north and lowest in the south.

The aims of this study were to determine the distribution of the ApoE genotype and how the status of ApoE4 is associated with the cognitive function of Korean health examinees with subjective memory complaints. Moreover, the cognitive domain scores were evaluated according to ApoE4 status.

Methods

Subjects

A retrospective cross-sectional study was conducted with 1,107 health examinees with subjective memory complaints who underwent both medical and cognitive testing including ApoE genotyping as part of a health checkup at two health-promotion centers in two Korean cities between January 2016 and December 2017. The exclusion criteria

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for this study were being previously diagnosed as AD or the presence of brain tumors, head trauma, or stroke. The study subjects gave their informed consent for cognitive testing and ApoE genotying.

Cognitive assessments

All subjects were assessed for cognitive function using the Korean version of Mini-Mental State Examination (K-MMSE) [14]. The MMSE is a widely used screening test for assessing cognitive abilities, and it was modified and translated into Korean to suit the cultural background of Koreans. The K-MMSE includes items assessing orientation (5 points for temporal orientation and 5 points for spatial orientation), registration (3 points), recall (3 points), attention and calculation (5 points), and language and praxis (2 points for naming, 3 points for oral command comprehension, and 1 point each for repetition, reading, writing, and visuospatial ability). The maximum K-MMSE score is 30, and higher scores indicate higher cognitive function. A K-MMSE score of ≤23 was defined as cognitive impairment [15].

ApoE genotyping

Whole peripheral blood was collected in EDTA tubes. Genomic DNA was extracted from the peripheral blood using SEEPREP 12 (DiaSorin, Dublin, Ireland) according to the manufacturers' protocol. ApoE was genotyped as recommended in the manufacturer's instructions using the Seeplex ApoE ACE Genotyping (Seegene, Seoul, Korea). Amplification has performed using TC-96/G/H(b)/A (Hangzhou Bioer Technology, Hangzhou, China) and the SEEAMP PCR System (Seegene, Seoul, Korea). Briefly, 3 μL of DNA extract in 17 μL of multiplex PCR Mastermix was amplified. ApoE genotypes were detected using the LabChip DX System (Caliper Life Sciences, Hopkinton, USA). The analysis yielded the following patterns: 158Cys and 112Cys for E2/E2; 158Cys, 112Cys and 158Arg for E2/E3; 158Cys, 112Arg, 112Cys and 158Arg for E2/E4; 112Cys and 158Arg for E3/E3; 112Arg, 112Cys and 158Arg for E3/E4; and 112Arg and 158Arg for E4/E4.

Statistical analysis

The study subjects were classified into ApoE4 non-carriers (E2/E2, E3/E3, and E2/E3), ApoE4 heterozygotes (E2/E4 and E3/E4), and ApoE4 homozygotes (E4/E4). Data are presented as mean±standard deviation or percentage for categorical variables. Analysis of variance (ANOVA) with Scheffe's multiple-comparison tests and the chi-square test were used to compare the characteristics according to the ApoE4 status. Univariate and multivariate (adjusted) logistic regression analyses were performed to evaluate the association between ApoE4 status and cognitive impairment. The multivariate models were implemented with adjustment for age, sex, body mass index (BMI), blood pressure, blood lipid levels, and fasting blood glucose level. ANOVA or Welch's ANOVA (in case of heterogeneity of the variance assumption) was used to compare the cognitive domain scores according to the ApoE4 status. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). A P value of <0.05 was considered statistically significant.

Results

These 1,107 examinees comprised 301 males and 806 females aged 66.2±8.3 years (range 38–89 years). The most frequent allele was ApoE3 (82.5%), followed by ApoE4 (10.6%) and ApoE2 (6.9%). The ApoE3/E3 genotype was the most common (68.4%), followed by ApoE3/E4 (17.2%), ApoE2/E3 (11.1%), ApoE4/E4 (1.4%), ApoE2/E4 (1.3%), and ApoE2/E2 (0.7%).

Characteristics of study subjects according to ApoE4 status

The overall K-MMSE score was 26.2±3.1 and was lower in ApoE4 homozygotes than in ApoE4 non-carriers (P=0.002). Cognitive impairment was more common in ApoE4 homozygotes than in ApoE4 non-carriers and ApoE4 heterozygotes (P=0.018). Blood triglyceride levels were higher in ApoE4 heterozygotes and ApoE4 homozygotes than in ApoE4 non-carriers (P=0.022) (Table 1).

Association of ApoE4 status with cognitive impairment

While age, sex, and systolic blood pressure were associated with cognitive impairment in a univariate model, this association of systolic blood pressure disappeared in multivariate models. ApoE4 homozygotes were significantly associated with cognitive impairment, and this association remained after adjusting for age, sex, BMI, blood pressure, blood lipid levels and fasting blood glucose level (odds ratio

Table 1. Characteristics of study subjects by ApoE genotype

	ApoE4 Negative		ApoE4 Heterozygote		ApoE4 Homozygote		Total		*P* value
	(N=888)		(N=204)		(N=15)		(N=1,107)		
K-MMSE	26.3	± 3[b]	25.7	± 3.4	24.1	± 4.2[a]	26.2	± 3.1	0.002
K-MMSE≤23 (N, %)	126	(14.2)	41	(20.1)	5	(33.3)	172	(15.5)	0.018
Age, yr	66.2	± 8.4	66.0	± 7.8	68.7	± 6.7	66.2	± 8.3	0.472
Male (N, %)	245	(27.6)	48	(23.5)	8	(53.3)	301	(27.2)	0.036
BMI, kg/m^2	24.5	± 3.2	24.5	± 3.2	23.2	± 2.1	24.5	± 3.1	0.281
SBP, mmHg	122.1	± 13.8	122.1	± 14.2	122.4	± 18.6	122.1	± 13.9	0.997
DBP, mmHg	74.2	± 8.4	73.5	± 9	75.2	± 8.2	74.1	± 8.5	0.577
TC, mmol/L	5.07	± 0.99	5.09	± 1.05	5.01	± 0.83	5.07	± 1.00	0.953
TG, mmol/L	1.21	± 0.73	1.39	± 0.77	1.25	± 0.48	1.25	± 0.74	0.022
HDL, mmol/L	1.50	± 0.37	1.43	± 0.36	1.38	± 0.42	1.49	± 0.37	0.057
LDL, mmol/L	3.01	± 0.89	3.04	± 0.98	3.09	± 0.78	3.01	± 0.9	0.880
FBS, mmol/L	5.68	± 1.34	5.86	± 1.34	5.58	± 1.78	5.72	± 1.35	0.278
HbA1c, mmol/mol	42.3	± 12.0	42.6	± 9.8	40.7	± 9.0	42.3	± 11.6	0.877

Data are mean±standard deviation or *N* (%) values.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, total triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FBS, fasting blood glucose; HbA1c, hemoglobin A1c.
P value derived from one-way ANOVA and chi-square test was used for intergroup comparison.
[a, b]: Different letters indicate a significant intergroup difference based on Scheffe's multiple-comparison test.

[OR]=4.651, 95% confidence interval [CI]=1.287–16.812). In ApoE4 heterozygotes, this association disappeared after adjusting for age, sex, BMI, blood pressure, blood lipid levels and fasting blood glucose level (Table 2).

Cognitive domain scores according to ApoE4 status

Table 3 presents the cognitive domain scores according to ApoE4 status. ApoE4 heterozygotes had significantly lower scores for temporal orientation (*P*=0.025) and recall domains (*P*=0.017) compared to ApoE4 non-carriers. In ApoE4 homozygotes, those cognitive domains showed lower scores compared to ApoE4 non-carrier and heterozygotes, but the differences did not reach statistical significance (Table 3).

Discussion

This study found that ApoE4 homozygosity was associated with cognitive impairment in health examinees with subjective memory complaints, and that this association persisted after adjusting for age, sex, and cardiovascular risk factors. Several domains of cognitive performance differed according to the ApoE4 status. ApoE4 heterozygotes had lower scores in the temporal orientation and recall domains compared to ApoE4 non-carriers.

The frequency of ApoE4 was 10.6% in our study. The prevalence of ApoE4 genotype has varied in previous studies. The human ApoE2, ApoE3, and ApoE4 alleles have worldwide frequencies of 8.4%, 77.9%, and 13.7%, respectively [16]. However, while the frequency of ApoE4 was 13.5% in an elderly Brazilian population [9], it was 8.7% in a community-dwelling Korean population [17]. Differences in the study subjects and methods used for ApoE genotyping may also have contributed to the variations in the reported prevalence rates of the ApoE4 genotype.

The effects of the ApoE genotype on the increased risk of AD are thought to be mediated by differential effects of ApoE4 on amyloid-β deposition in the brain [18]. The general effects of ApoE4 that result in cognitive deficits also include ApoE4-related impairment to neuronal integrity and repair mechanisms as well as cardiovascular-related effects of the ApoE4 genotype [19]. ApoE4 carriers are at an increased risk of not only AD associated with an increased risk of cerebral amyloid angiopathy, but also age-related cognitive decline during normal aging [20]. Healthy ApoE4 carriers who are not diagnosed with mild cognitive impairment (MCI) or AD show accelerated longitudinal declines in memory tests, revealing the possibility of a pre-MCI state in ApoE4 carriers [21]. This association of ApoE4 with cognitive decline showed many years before cognitive impairment becomes clinically apparent in study using a mixed model for longitudinal change with age [22]. In cognitively normal people, ApoE4 is associated with enhanced amyloid pathology, which increases the amount of amyloid deposition, in a gene-dose-dependent manner [23]. A previous meta-analysis [24] found that cognitive impairment was associated with ApoE homozygosity but not heterozygosity. In our study, ApoE4 homozygosity was associated with cognitive impairment, and this association persisted after adjusting for age, sex and cardiovascular risk factors. However, this association with cognitive impairment in ApoE4 heterozygotes disappeared after adjusting for cardiovascular risk factors such as BMI, blood pressure, fasting blood glucose level and blood lipid levels. These findings suggest that there could be the gene-dose-dependent manner of relationship between ApoE4 genotype and its effect on cognitive impairment.

The effect of ApoE4 on the cognitive performance domains was selective in our study. Several studies [25,26] have observed decrements in episodic memory function among ApoE4 carriers, with a suggested underlying mechanism being ApoE4 carriers having a smaller hippocampal volume. Moreover, Yip et al. [27] reported that

Table 2. Association between ApoE4 status and cognitive impairment (K-MMSE score≤23)

	Univariate model		Multiple model			
			Model 1		Model 2	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Allele E4(ref=0)						
1	1.521	(1.029, 2.249)	1.498	(0.938, 2.394)	1.507	(0.933, 2.435)
2	3.024	(1.017, 8.993)	3.887	(1.143, 13.221)	4.651	(1.287, 16.812)
Age, yr	1.14	(1.112, 1.169)	1.139	(1.107, 1.172)	1.14	(1.107, 1.174)
Sex(ref. male)	1.494	(1.007, 2.215)	2.147	(1.326, 3.479)	2.205	(1.339, 3.632)
BMI, kg/m^2	0.983	(0.928, 1.041)	0.974	(0.913, 1.039)	0.961	(0.897, 1.029)
SBP, mmHg	1.027	(1.014, 1.04)	1.013	(0.992, 1.033)	1.012	(0.991, 1.033)
DBP, mmHg	1.019	(0.998, 1.041)	0.999	(0.967, 1.033)	1.002	(0.968, 1.036)
TG, mmol/L	1.033	(0.817, 1.307)			0.93	(0.65, 1.329)
HDL, mmol/L	0.676	(0.412, 1.11)			0.613	(0.319, 1.178)
LDL, mmol/L	0.924	(0.757, 1.129)			1.036	(0.83, 1.293)
FBS, mmol/L	1.077	(0.958, 1.21)			1	(0.855, 1.169)

Data are odds ratio (OR) and 95% confidence interval (CI) values.
Model 1: Adjusted for age, sex, and body mass index (BMI), and blood pressure.
Model 2: Adjusted for age, sex, BMI, blood pressure, blood lipid levels, and fasting blood glucose level.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, total triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FBS, fasting blood glucose.

Table 3. Cognitive domain scores according to ApoE4 status

Domains	ApoE4 non-carriers (N=888)		ApoE4 heterozygotes (N=204)		ApoE4 homozygotes (N=15)		*P* value
TemporL orientation*	4.70	± 0.71[a]	4.5	± 1.02[b]	4.4	± 1.4	0.025
Spatial orientation*	4.56	± 0.63	4.48	± 0.74	4.2	± 0.77	0.106
Registration	2.96	± 0.25	2.95	± 0.24	3	± 0	0.698
Attention and calculation	3.67	± 1.39	3.69	± 1.39	3.07	± 1.67	0.243
Recall*	1.84	± 0.97[a]	1.62	± 1.01[b]	1.4	± 1.3	0.017
Language and praxis*	8.57	± 0.75	8.48	± 0.9	8	± 1.2	0.102

Data are mean±standard deviation. *Using Welch's ANOVA in case of heterogeneity of the variances assumption. [a,b]Different letters indicate a significant difference

other ability domains such as primary memory (short-term memory) and visuospatial function were less affected among ApoE4 carriers. These findings are consistent with our results, in which ApoE4 carriers exhibited lower scores in the temporal orientation and delayed recall domains compared to ApoE4 non-carriers, but with no significant decrease in short-term memory domains such as registration and other domains. Contrary to our expectations, although the temporal orientation and delayed recall domains showed the lowest scores in ApoE homozygotes, the differences did not reach statistical significance. This lack of significance could have been due to the relatively small number of ApoE4 homozygotes.

A prospective study of a cognitively normal cohort showed that the risk of dementia in ApoE4 carriers is negatively associated with higher education, higher level of leisure activities, and the absence of vascular risk factors [28]. Moreover, Head, et al. [29] demonstrated that physical exercise is strongly associated with reduced positivity for Pittsburgh compound B–which indicates the presence of fibrillar aggregates of amyloid-β–in cognitively normal ApoE4 carriers. Those studies indicate that higher education, actively participating in leisure activities and exercise, and maintaining vascular health could be beneficial in reducing the risk of AD and cognitive decline, particularly among ApoE4 carriers. Although the ApoE genotype is an unmodifiable factor of cognitive impairment, knowledge of which ApoE genotype a person is carrying could be helpful in prevention or delaying cognitive impairment by promoting health behaviors in a targeted manner.

This study has some limitations. First, the cross-sectional design meant that the study could not reveal the causal relationship between ApoE genotypes and cognitive function. Also, potential confounding factors for cognitive function such as education, and the presence of depression were not adjusted. However, age, sex, and cardiovascular risk factors including BMI, blood pressure, blood lipid levels, and fasting blood glucose level were adjusted when evaluating the association between ApoE4 status and cognitive impairment. Second, cognitive function was measured using single cognitive measure, the K-MMSE. However, the K-MMSE is the most commonly used screening test in primary care units, including health-promotion centers. Finally, the preponderance of female participants (73%) is another limitation of our study.

Conclusion

In conclusion, the present study involving health examinees with subjective memory complaints found that ApoE4 homozygosity was associated with cognitive impairment, after adjusting for age, sex and cardiovascular risk factors. ApoE4 carriers had significantly lower scores for temporal orientation and recall domains compared to ApoE4 non-carriers. Our results suggest that ApoE4 carriers with subjective memory complaints should receive close monitoring for the development of AD and recommendations to exercise and maintain vascular health in order to prevent or delay cognitive impairment.

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Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

Ethics statement

This study was approved by the Institutional Review Board of the Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

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