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APOE Genotype in the Diagnosis of Alzheimer's Disease in Patients With Cognitive Impairment

Xiaoyan Sun, MD, PhD[1], Joyce Nicholas, PhD[1,2], Aljoeson Walker, MD[1], Mark T. Wagner, PhD[1] and David Bachman, MD[1]

Abstract

Background: Although an association between the apolipoprotein E (*APOE*) ε4 allele and increased risk of Alzheimer's disease (AD) is established, the utility of *APOE* genotyping in the clinical diagnosis of AD is still under investigation. **Methods:** Medical records of 89 patients with cognitive impairment and *APOE* genotype data underwent a retrospective review. **Results:** Comparison of age, age at onset, education, Mini-Mental State Examination, months of follow-up, and family history of dementia did not reveal statistical difference among the patients with different *APOE* genotypes. The *APOE* ε4 carriers had a higher percentage of AD diagnoses after a median 16 months follow-up than non-*APOE* ε4 carriers. The *APOE* ε4 designation had a high sensitivity and high positive predictive value for the diagnosis of AD but a low negative predictive value and specificity. **Conclusions:** The *APOE* genotyping may be helpful in diagnosing AD especially in patients presenting with atypical features or early age of onset of dementia.

Keywords

Alzheimer's disease, *APOE*, *APOE* ε4, sensitivity, positive predictive value

Introduction

A strong association between the *APOE* (apolipoprotein E) ε4 allele and increased risk of Alzheimer's disease (AD) is well established based on extensive clinical and basic studies.[1,2] It is reported that *APOE* ε4 carriers have an increased risk of late onset of AD of 3 to 15 times in a gene dose-dependent manner.[3] The *APOE* ε4 carriers have an earlier age of onset of AD by 10 to 20 years as compared to the noncarriers.[4]

The mechanism of *APOE* ε4 allele in the pathogenesis of AD is not entirely understood. Both amyloid-β (Aβ)-dependent and Aβ-independent roles for *APOE* ε4 allele in the pathogenesis of AD have been proposed.[2,5] The presence of *APOE* ε4 allele is associated with increased Pittsburgh compound B binding and amyloid deposition in in vivo and postmortem human studies.[1,6-8] The correlation between reduced cerebral spinal fluid (CSF) Aβ 42 and *APOE* ε4 allele has also been reported.[6] Regarding the amyloid-independent role of *APOE* ε4 allele in the development of AD, recent functional magnetic resonance imaging (fMRI) studies report that *APOE* ε4 allele disrupts the resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Aβ 42.[9,10] Earlier study shows the mean normalized hippocampal right and left hippocampal volumes are smaller in the ε4 carriers versus noncarriers with normal cognition. Greater medial temporal lobe atrophy is reported in the patients with AD who are ε4 carriers although conflicting data exist.[11-14] Taken together, it is suggested that *APOE* ε4 allele plays a vital role in the pathogenesis of AD.

Although *APOE* genotyping is not recommended for use alone in routine clinical diagnosis, a few studies have reported on a possible role of *APOE* genotyping in actual clinical practice.[15-17] In the present study, we undertook a retrospective analysis of *APOE* genotype in the patients referred to a memory disorders clinic in a university hospital setting. Comparison of the clinical diagnosis at first visit was made with the clinical diagnosis at follow-up visit after the *APOE* genotype information was available. Using the diagnosis of AD at the follow-up visit as our outcome measure, we calculated the positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of *APOE* genotype testing.

[1] Department of Neuroscience, Medical University of South Carolina, Charleston, SC, USA
[2] Department of Medicine, Medical University of South Carolina, Charleston, SC, USA

Corresponding Author:
Xiaoyan Sun, MD, PhD, Department of Neuroscience, Medical University of South Carolina, 96 Jonathan Lucas Street, Charleston, SC 29403, USA
Email: xns@musc.edu

Methods

Participants

This retrospective study was performed at the Medical University of South Carolina (MUSC), with approval by institutional review boards. The charts dated January 2005 to November 2010 of the 335 patients referred to the memory disorders clinic were reviewed. Those patients carried *International Classification of Diseases, Ninth Revision, Clinical Modification* diagnostic code 290 (dementia), 294.8 (dementia, not otherwise specified), and 331.83 (mild cognitive impairment [MCI]). A total of 89 patients (26.6% of the total referred patients) with these diagnostic codes had *APOE* genotyping performed subsequent to the first visit. The *APOE* genotyping was performed in those patients if the patients had an atypical presentation or early age of onset of cognitive impairment. The patients were evaluated by 2 senior neurologists (DB or AW) including detailed history, neurological examination, and routine laboratory analysis. If brain imaging was not already available, it was also obtained. In the present study, other specialized diagnostic studies such as flurodeoxyglucose F 18 positron emission tomography (PET) scanning or CSF studies of Aβ 42 and p-tau were not considered for an initial clinical diagnosis. Dementia diagnosis was established using *Diagnostic and Statistical Manual of Mental Disorders* (Third edition) criteria.[18] Clinical AD diagnosis was established using National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for possible or probable AD.[19] The initial diagnosis was based on the history and clinical assessment at the first visit prior to *APOE* genotyping. The follow-up diagnosis was obtained from the last available clinical note that was based on the clinical assessment which included all available clinical and laboratory data as well as routine imaging studies.

APOE Genotypes and Statistical Analysis

Approximately 10 mL of blood were collected from each patient in EDTA tube, gently mixed by inversion and shipped at room temperature to Athena Diagnostic (Athena Neurosciences Inc, Worcester, MA) by the laboratory of MUSC hospital within 24 hours of collection for the *APOE* genotype analysis.[17,20] The *APOE* ε4 positive designated as the participants carry at least 1 ε4 allele. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS, version 17) software (SPSS Inc, Chicago, Illinois). The Student's 2-sided *t* test for independent samples was used for continuous variables (Mann-Whitney *U* for nonparametric continuous) and chi-square (χ^2) for categorical variables. A *P* value less than .05 was considered statistically significant. Allele frequencies were determined by counting alleles. The agreement of the observed genotype frequency with expected genotype frequency according to the Hardy-Weinberg equilibrium was verified by χ^2 test. Sensitivity was defined as the percentage of AD cases who were ε4 positive; specificity was defined as the percentage of non-AD cases who were ε4 negative; and PPV was defined as the percentage of individuals who were ε4 positive who had the diagnosis of AD after follow-up. The median follow-up time was 16 months.

Table 1. Demographic Data of the Patients[a]

Variables	
Age, y (N = 89)	65.0 ± 9.4
Age at onset, y (N = 89)	62.3 ± 9.6
Education, y (N = 57)	14.5 ± 3.0
Female (%)	58 (65)
Caucasian (%)	75 (84.3)
Africa American (%)	12 (13.5)
Asian (%)	1 (1.1)
Other (%)	1 (1.1)
APOE ε4 (%)	70.8
Positive family history of dementia, % (N = 86)	58
MMSE (N = 78)	21.6 ± 5.9
Months follow-up (N = 89)	16 (1, 72)

Abbreviations: *APOE*, apolipoprotein E; MMSE, Mini-Mental State Examination; SD, standard deviation.
[a] Age, age at onset, education, and MMSE score are expressed as mean ± SD; months follow-up is expressed as median (minimum and maximum).

Table 2. The *APOE* Genotypes and Allele Frequency Among the Patients

APOE genotype	N (%)	*APOE* allele	Distribution of *APOE* allele frequency
2/3	5 (5.6)	ε2	0.04
3/3	21 (23.6)	ε3	0.47
2/4	2 (2.2)	ε4	0.49
3/4	37 (41.6)		
4/4	24 (27)		

Abbreviation: *APOE*, apolipoprotein E.

Results

Demographic Features of the Patients

Data on 89 patients referred to the memory clinic for evaluation of cognitive impairment were collected in this study for those patients that had *APOE* genotypes testing and at least 1 follow-up visit from 2005 to 2011. The average age at the initial visit was 65 years (standard deviation [SD] 9.4) and age at onset was 62.3 years (SD 9.6). Among all patients, there were 57 patients (64%) who developed symptoms before 65 or at 65 years. Ten patients (11%) presented with dominant behavior and mood symptoms including agitation, irritability, depression, anxiety, hallucination, paranoia, and confusion in the context of memory impairment. Six patients (7%) exhibited relatively stable clinical course of memory impairment. Sixteen patients (18%) had differential diagnosis of normal pressure hydrocephalus, frontotemporal lobe dementia, neurosarcoidosis, seizure, vascular dementia, cognitive impairment after cardiac events, dementia unresponsive to B12, and heavy metal chelation treatment. In this sample, 58 participants

Table 3. Comparison of Different Variables Between the ε4 Noncarriers and ε4 Carriers[a]

APOE genotypes	Age, y	Age at onset	Education	MMSE	Months follow-up	Family history of dementia
2/3 (n = 5)	65.4 ± 6.1	62.6 ± 8.0	12.0 ± 0.0	21.5 ± 5.7	15 (1, 72)	100.0%
3/3 (n = 21)	61.4 ± 9.2	59.4 ± 9.09	14.5 ± 2.9	22.7 ± 4.5	12 (1, 72)	42.1%
2/4 (n = 2)	54.0 ± 1.4	52.5 ± 2.1	12.0 (n = 1)	25.0 ± 2.8	21 (18, 24)	0.0%
3/4 (n = 37)	67.1 ± 10.7	64.5 ± 11.5	14.9 ± 3.2	21.9 ± 6.1	12 (2, 60)	67.6%
4/4 (n = 24)	65.5 ± 7.0	62.1 ± 7.5	14.5 ± 2.9	20.1 ± 6.6	18 (1, 72)	54.2%
P value of ε4 versus non-ε4 carriers	.07	.16	.56	.46	.48	.62

Abbreviations: *APOE*, apolipoprotein E; MMSE, Mini-Mental State Examination; SD, standard deviation.
[a] Values are mean ± SD except month follow-up expressed as a median (minimum and maximum).

Table 4. Clinical Diagnosis of the Patient in Association With *APOE* Genotypes

	Initial diagnosis				Follow-up diagnosis			
APOE genotype	AD	MCI	Non-AD	Total	AD	MCI	Non-AD	Total
2/3 (n = 5)	1	2	2	5	3	0	2	5
3/3 (n = 21)	6	4	11	21	10	2	9	21
2/4 (n = 2)	0	0	2	2	0	0	2	2
3/4 (n = 37)	14	14	9	37	24	8	5	37
4/4 (n = 24)	13	5	6	24	23	1	0	24
P value of ε4 versus non-ε4 carriers	.11				<.01			

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; MCI, mild cognitive impairment.

(65%) were female. There were 75 Caucasian (84.3%), 12 Africa American (13.5%), 1 Asian (1.1%), and 1 other (1.1%). The *APOE* ε4 carrier status was positive in 71% of the participants. In this sample, 58% of the cases had a positive family history of dementia. There were 3 participants without known family history. Two of them were adopted. One of them was not documented in the chart. The average Mini-Mental Status Examination (MMSE) score at the first visit was 21.6 for 78 cases (SD = 5.9). The scores were not adjusted for education and age. Some of participants did not undertake MMSE test either due to severely impaired or highly functional cognition. Median follow-up duration was 16 months (Table 1).

Different Variables Among the Patients With Different APOE Genotype

The *APOE* genotype and allele frequency distributions in the patients are given in Table 2. There are 41.6% *APOE* ε3/4, 27% *APOE* ε4/4, 2.2% *APOE* ε2/4, 23.6% *APOE* ε3/3, and 5.6% *APOE* ε2/3 (Table 2). The allele frequencies are reported as ε2 = 0.04, ε3 = 0.47, and ε4 = 0.49. All the observed allele frequencies fits those expected allele frequencies according to Hardy-Weinberg equilibrium ($P = .49$). Table 3 shows the age at the initial visit, age at onset, education, MMSE, follow-up duration, and family history of dementia by *APOE* genotype among the patients with different *APOE* genotype. There are no statistical differences between these variables among the patients across different *APOE* genotype. There are no statistical differences between these variables between *APOE* ε4 carriers and non-ε4 carriers. Table 4 shows the comparison between initial diagnosis and a follow-up diagnosis of AD in association with *APOE* genotype. There are no statistical differences in the clinical diagnosis of AD between ε4 and non-ε4 carriers at the initial visit. There is, however, a statistical difference in the AD diagnosis between ε4 and non-ε4 carriers after a median of 16 months follow-up.

The PPV, NPV, Sensitivity, and Specificity of APOE ε4 in Association With a Clinical Diagnosis

The PPV, NPV, sensitivity, and specificity of *APOE* ε4 in association with clinical diagnosis of AD are calculated. The PPV is 77%; NPV 52.4%; sensitivity 82.5%; and specificity 44%.

Discussion

The *APOE* genotype testing has been used primarily as a clinical research tool to evaluate the risk population of AD. The utility of *APOE* genotyping in the clinical diagnosis of AD is still under investigation.[21,22] A recent clinical trial of monoclonal antibody directed against Aβ suggests that *APOE* ε4 carriers may have a different response to amyloid removal therapy than noncarriers. Noncarriers show a treatment-associated response on cognitive scores. By contrast, patients with 1 or 2 alleles of *APOE* ε4 show no treatment effect on any measure. In addition, *APOE* ε4 carriers had more vasogenic edema.[23] Taken together, it is suggested that stratifying *APOE* by genotype may be useful in clinical practice if disease-modifying therapy is available.

Table 5. Comparison of Sensitivity, Specificity, PPV, and NPV of *APOE* ε4 in the Diagnosis of AD Among Various Studies

	Saunders et al,[15] N = 67[a]	Tsuang et al,[16] N = 132[a]	Welsh-Bohmer et al,[17] N = 162[a]	Slooter et al,[30] N = 249[b]	Mayeux et al[21]; McConnell et al,[29] N = 2188[a]	Kakulas et al,[28] N = 66	Smith et al,[31] N = 115	Current study, N = 89[b]
Frequency of *APOE* ε4	64%	50%	71%	34%	69%	38%		71%
Sensitivity	75%	59%	83%	32%	65%	46%	78%	82.5%
Specificity	100%	71%	83%	61%	68%	100%	100%	44%
PPV	100%	83%	97%	69%	90%	100%	100%	77%
NPV	42%	41%	44%		31%		0	52.4%
Age at diagnosis, y		80.7 + 7			72 + 10			65 ± 9.4
Age at death, y		83 ± 7	77.2 ± 7.8	83.5 ± 7.3	77 ± 10			

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; NPV, negative predictive value; PPV, positive predictive value.
[a] The AD was diagnosed by pathological criteria.
[b] The AD was diagnosed with clinical criteria.

In our memory disorders clinic, we do not perform the *APOE* genotyping for the routine diagnosis of AD. We obtain the *APOE* genotypes only in those patients who have an early age of onset of cognitive impairment or present with atypical symptoms of presumed AD. This is a retrospective study to evaluate the role of *APOE* ε4 in the clinical diagnosis of AD in this selected population. To compare our patient population with those from other studies, we calculate the frequency of *APOE* ε4 allele. The frequency of *APOE* ε4 (49%) allele in the patients of the present study is higher than the result obtained in a large meta-analysis of 5107 Caucasian with AD (36.7%).[3] The discrepancy is mostly due to age-dependent variation. It is reported that the frequency of *APOE* ε4 allele is higher in those patients with age at onset as 60 to 69 years. The frequency of *APOE* ε4 allele (49%) in the present study is similar to the results obtained from other studies of patient with AD in this age group.[24,25] Using the *APOE* ε4 allele-enriched patient group, we are likely to observe the maximal effect of the ε4 allele in the development of AD.[26] In this cohort, the average age of the patients at onset was younger than 65 years. We found significantly more AD cases among *APOE* ε4 carriers than noncarriers, confirming that *APOE* ε4 is strongly associated with AD in our clinical setting. As Poirier et al reported, analysis of ε4 allele frequency as a function of age revealed a bimodal distribution, with peaks at 65 (55-65 years old) and 75 years (75-85 years old) in AD.[27] Taken together, it is suggested that *APOE* ε4 might accelerate neurodegenerative process both in an aging-dependent and an independent manner.[27] Clinically, if a patient with early age of onset of memory impairment has positive *APOE* ε4, he or she is likely to be diagnosed with AD in the course of disease. To our knowledge, there are no studies to evaluate the role of *APOE* genotyping in the diagnosis of clinically challenging cases.

Comparison of our study with others reveals that high PPV (69%-100%) and low NPV (0%-52.4%) of *APOE* ε4 in the diagnosis of AD are the consistent findings across all the studies regardless of the age of the patients and the frequency of *APOE* ε4[15-17,21,28-31] (Table 5). Our result together with other studies suggests that the presence of *APOE* ε4 allele in patients with cognitive impairment has a high probability of predicting the future development of AD in these individuals. At follow-up visits in our study, more *APOE* ε4 carriers were eventually diagnosed as having AD. This occurred because many patients carrying other clinical diagnoses were rediagnosed as having AD. However, many non-ε4 carriers were also diagnosed as having AD. Therefore, the absence of the ε4 allele did not preclude an eventual diagnosis of AD. It remains to be seen whether the excellent PPV mainly found at hospital memory disorder clinic setting would be applicable to other community settings. One could argue that the PPV might actually be higher since the prevalence of AD among all dementia cases is likely to be higher in the community than at a specialized memory disorders clinic where one would expect to see more unusual etiologies of dementia. Regarding sensitivity, there are differences among the studies. Low sensitivity is mostly observed in the patients who are older[16,28,30] or in data obtained from multiple centers.[21]

Limitations of the current study include a relatively small sample size, and the final clinical diagnosis as the outcome rather than the pathological diagnosis. However, a median of 16 months follow-up did allow for the natural progression of the patient's symptoms to play an important role in assigning a final diagnosis. A high conversion rate to AD (16%-64%) has been observed in several other studies after 1 to 2 years follow-up of patients with MCI.[32-34]

As our samples were obtained from retrospective study, the observers were not blinded to the *APOE* genotype status. However, the follow-up diagnosis was established in combination with MRI of the brain, laboratory tests, PET scan, and CSF amyloid and tau studies at the discretion of the treating neurologist. Therefore, *APOE* genotype was not the sole consideration in the final diagnosis, though an important consideration. In general, the follow-up of the natural progression of symptoms was the major factor in determining the final diagnosis. Further prospective studies with larger sample sizes are needed to validate our findings.

In the past, the clinical diagnosis of AD was largely a diagnosis of exclusion. Recently, research has demonstrated that the temporal unfolding of biological markers will likely play an increasingly important role in understanding the pathophysiology of AD. Very likely, biological markers and risk factor genes will be more frequently utilized in the characterization of individual patients in the clinical setting for future available treatment.[35]

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Declaration of Conflicting Interests

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