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APOE4 STATUS AND COGNITIVE FUNCTION IN MIDDLE-AGED AND ELDERLY PEOPLE

Pavel N.A.[1], Paun M.R.[2], Matei P.V.[1,2], Dutu I.[1], Tudose C.[1,2]

[*]**Corresponding Author:** Valentin P. Matei, Associate Proffesor, MD, PhD. 2nd Ward of "Prof. Dr. Alexandru Obregia" Hospital, Berceni Street no 10, Bucharest, Romania. E-mail: valipmatei@yahoo.com

ABSTRACT

Introduction: *APOE* is one of the prominent genes involved in the increased risk of developing Alzheimer's disease, but its effect on cognition in patients who are not yet diagnosed with dementia or mild cognitive impairment is relatively understudied. We aimed to examine the effect of ApoE4 on cognitive performance in unimpaired middle-aged and elderly persons.

Materials and methods: Our study included 51 cognitively unimpaired participants divided into ApoE4 positive patients and controls by *APOE* genotyping. The following clinical and demographic characteristics were collected: age, gender, education, social status, BMI, history of medical or psychiatric disorders. Patients with current anxiety or depressive disorders were excluded. Cognitive function was evaluated using MMSE, Rey Auditory-Verbal Learning Test, Rey Complex Figure test, TMT A and B and verbal fluency test. The two groups were matched for age, sex, and education. Categorial data was analyzed using Chi-Square and continuous data using Student-T test (parametric variables) or Mann-Whitney test (non-parametric variables). Statistical significance was considered at $p \leq .05$.

Results: There were 11 (21.6%) ApoE4 positive patients and 40 (78.4%) controls. There were no significant differences between the groups regarding socio-demographic and clinical characteristics. The ApoE4 positive group performed slightly worse on cognitive evaluations compared to controls but only the mean scores of the Rey Complex Figure Test – Memory reached statistical significance ($p=.019$).

Conclusion: Cognitive evaluation generally rendered lower scores in the ApoE4 group compared to the control group. However, only visual memory impairment scores were significantly lower in the ApoE4 positive individuals than in controls.

Keywords: *APOE*, cognitive impairment, elderly, subjective cognitive decline, SCD.

INTRODUCTION

The *APOE* gene encodes one of the major protein families involved in lipid metabolism, named apolipoproteins [1]. It is situated on the long arm of chromosome 19, and is most frequently found in isoforms resulting from permutations of three alleles: ε2, ε3 and ε4 [1], their relative frequency in the Caucasian population being approximately 8%, 74% and 14%, respectively [2].

The presence of the ε4 allele, both in hetero- or homozygote form, is one of the most well-known risk factors for Alzheimer disease (AD), and accounts for about 20-25% of heritable susceptibility [3]. In individuals suffering from AD, its presence is associated with increased amyloid plaque density [4] as well as the accentuation of a number of non-specific neurodegenerative processes [5], which translate into a particular disease phenotype characterized by greater atrophy of the parietal and temporal lobes and more severe memory deficits compared to ApoE4 negative AD patients [6].

The detrimental effects of ApoE4 are not limited to increased risk and severity of AD; but also extend to a significant degree to clinically unimpaired carriers. Morphologically, these individuals exhibit lower grey matter volumes in several brain regions frequently affected in AD patients, most notably the hippocampus, frontal cortex and thalamus [7]. ApoE4 is also associated with neuronal hypometabolism in the cingulate cortex [8], as well as increased permeability of the blood-brain barrier in the

[1] Psychiatry Department, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

[2] "Prof. Dr. Alexandru Obregia" Psychiatric Hospital, Bucharest, Romania

hippocampus and temporal lobe [9]. Functionally, ApoE4 positive individuals appear to perform worse in memory and attention related tasks as early as middle age [10].

The aims of our study are to examine the effect of ApoE4 status on cognitive performance in a cohort of clinically unimpaired elderly patients, divided into an ApoE4 positive and an ApoE4 negative group after genotyping. The groups are matched by age, socioeconomic status, educational attainment, and psychiatric and somatic illnesses. In doing so, we hope to better understand the value of these parameters, as predictors for neurodegenerative disorders and to better understand the impact of ApoE4 prior to the onset of these disorders.

MATERIAL AND METHODS

We conducted a cross-sectional study on patients from a primary care clinic who were recruited while performing their clinical routine checkup at their General Practitioners (GPs).

The study was conducted in accordance with the Declaration of Helsinki [11] and was approved by the Local Ethics Committee (no. 11/06/03.2020). Patients included in the study had to understand and sign the Informed Consent Form and fulfill all the described criteria: (a) age between 50-80 years, (b) Mini-Mental State Examination (MMSE) score over 24, (c) Functional Assessment Questionnaire (FAQ) below 9, (d) Hamilton Depression total score below 12, (e) Hamilton Anxiety total score below 17 and (f) no substance use in the previous 6 months other than caffeine or tobacco. Exclusion criteria were: (a) diagnosis of major or mild neurocognitive disorder according to DSM 5,[12] (b) presence of cerebro-vascular disease translated as Hachinski score over 4, (c) current diagnosis of neurodevelopmental disorder, major depressive disorder, anxiety disorder according to DSM 5, (d) severe somatic disorders such as epilepsy, organ failure or other disease that could impair collection of data from the patient such as severe hearing/seeing impairment, motor deficit).

We collected social, demographic, and clinical information from the participants, as well as their full psychiatric history.

For genetic assessment of *APOE* status, we collected 3 milliliters of venous blood using EDTA coated tubes, which were then kept refrigerated at 4 degrees Celsius until DNA extraction, which was conducted using DNA extraction kits (Wizzard© Genomic DNA Purification Kit, Promega) form a volume of 300 μL white blood cells. The genotyping was done using the Taqman SNP Genotyping Assay method from Thermo Fished Scientific, according to the manufacturer's instructions. The assay was conducted by PCR amplification of two oligonucleotide primers, rs429358 and rs7412. Patients with at least one ApoE4 allele were considered ApoE4 positive while the rest were coded as ApoE4 negative and were considered as control group.

Cognitive examination was conducted using the MMSE [13], Rey Auditory-Verbal Learning Test (RAVLT) [14], Rey-Osterrieth Complex Figure Test [15], Verbal Fluency [16] and Trail Making Test (TMT) [14].

The Mini-Mental State Examination [13] is a short questionnaire that evaluates attention, memory, calculation, visuo-spatial ability and executive functioning. The maximum score is 30 and scores under 24 are considered suggestive for cognitive impairment.

The RAVLT [14] consists of a list of 15 words, and is designed to evaluate the ability to assess verbal memory. The evaluator reads a list of 15 words after which the participant is asked to recall as many as he can; this is done for a total of 5 times (Trials 1-5). Afterwards, the same exercise is done with a different list of 15 words for a single trial and the participant is then asked to recall the words from the first list (Trial 6). Trial 7 (Delay) is done after a 5-minute pause, without the list being reread to the patient. The last examination of RAVLT (Recognition) consists of a text which includes the first list of words, and the patient is asked to recognize them.

The Rey-Osterrieth Complex Figure Test [15] consists of 2 trials which examine memory, visuo-spatial ability and executive function. For the first trial (copying), the patient is asked to copy a complex figure, after which the figure is put away. Following a 3-minute break the patient is asked to draw the figure from memory. The maximum score is 36 with higher scores representing better cognitive functioning.

The Verbal Fluency [16] test examines verbal ability and executive function. We used the letter fluency variant, consisting of 3 successive 1-minute trials, in which the patient must produce as many words as possible that begin with a given letter. Each trial used a different letter, and all patients received the same letters in the same order. In our analysis we summed all the correct words from all trials into a single score.

The Trail Making Test [14] examines a variety of cognitive functions such as attention, visual and spatial ability, sequencing and shifting, psychomotor speed, abstraction, flexibility and executive function [14]. It consists of two timed trials (A and B). Patient is considered to be deficient in the presented cognitive domains if he finishes Trial A after 78 second and Trial B after 273 seconds.[17]

The ApoE4 and control groups were matched for age, sex and education and there were no patients with current depressive or anxiety disorder. Descriptive statistics were used to present the sample characteristics. We used

Table 1. Socio-demographic and clinical characteristics

Item	ApoE4 (N=11)	Controls (N=40)	p
Gender (Female)	7 (63.6%)	34 (85%)	.193
Age	65.64 (8.02)	64.75 (7.19)	.725
Education (years) – Mean (SD)	13.18 (3.49)	13 (3.20)	
Median (IQR)	13 (12-16)	13 (10.25-16)	.872
From Urban areas	7 (63.6)	24 (60)	.827
In a relationship	5 (45.5)	22 (55)	.574
BMI – Mean (SD)	27.98 (5.08)	28.66 (4.61)	
Median (IQR)	25.71 (23.44-34.24)	27.82 (25.31-31.82)	.544
Hypertension	8 (72.7)	26 (65)	.731
Type II Diabetes	1 (9.1)	8 (20)	.663
Currently pharmacological treatment for somatic disorders	8 (72.7)	34 (85)	.385
History of Psychiatric disorders	5 (45.5)	10 (25)	.187
HAM-D score, Mean (SD)	2 (2.28)	2.2 (1.59)	
Median (IQR)	1 (0-3)	2 (1-3)	.393
HAM-A score, Mean (SD)	2.64 (2.34)	2.95 (2.14)	
Median (IQR)	2 (0-4)	2 (2-4)	.699

HAM-D – Hamilton Depression Scale, HAM-A – Hamilton Anxiety Scale; Education, BMI, HAM-D Score, HAM-A score were analyzed using Mann-Whitney; Age was analyzed using Student-T Test; Gender, From Urban Areas, In a relationship, Hypertension, Type II Diabetes, Currently pharmacological treatment for somatic disorders, history of psychiatric disorders was analyzed using Chi-Square.

Chi-square test for the categorical data such as (ApoE4 status, demographic characteristics) and Student t-test (for normal distributed variables) and Mann-Whitney Test (for non-parametric variables) for continuous variables (such as age, cognitive evaluation test scores). Results are presented with mean (standard deviation) for normally distributed data and median (Inter Quartile Range - IQR) for non-parametric distribution. Statistical significance was considered at alpha below .05.

RESULTS

There were 51 patients included in the study, 11 ApoE4 positive (21.6%) and 40 controls (78.4%). There were more female patients in each group (63.6% female and 36.4% male in the ApoE4 positive group and 85% female and 15% male in the control group), and the mean age for the ApoE4 group was 65.64 years compared to 64.75 in the control group. There were no statistically significant differences between the groups regarding locative status, relationship status, BMI, hypertension, type II diabetes, current treatment use, history of psychiatric disorders, HAM-D and HAM-A scores. The fully described results of the socio-demographic and clinical characteristics of participants with ApoE4 compared to controls are presented in Table 1.

There were no statistically significant differences regarding cognitive functioning between the ApoE4 positive group and controls, except in the Rey Complex Figure Test - Memory (p=.019). The ApoE4 positive group exhibited a trend of poorer performance than the control group in most tasks. The full results of the cognitive evaluations are presented in Table 2.

DISCUSSION

The goal of our study was to analyze the impact of ApoE4 status on various cognitive domains in 51 individuals that did not screen positive for cognitive impairment on the MMSE test or clinical evaluation. The prevalence of ApoE4 in our cohort was 21.5%, which is higher than the 14% generally estimated in the Caucasian population [2].

Our main result was that ApoE4 positive (ApoE4+) group scored significantly worse than the ApoE4 negative (ApoE4-) group in the Rey Visuospatial Memory Test. Secondly, ApoE4+ individuals exhibited a negative trend in all other cognitive tests, most notably in the RAVLT Trial 5 and RAVLT Recognition tests, in which the difference was just short of statistical significance.

This corroborates with previous data that associate ApoE4 with lower-than-expected performance in general memory and attention driven tasks, even in middle-aged individuals [18]. Additionally, deficits in spatial cognition were identified in ApoE4 positive mice from a young age [19]. Impairments in visuospatial memory are often present during the early stages of AD or in Mild Cognitive

Table 2. Cognitive evaluation of ApoE4 positives vs controls

Item	ApoE4 positive (N=11)	Controls (N=40)	p
MMSE			
Mean (SD)	28.46 (1.81)	28.35 (1.33)	
Median (IQR)	29 (27-30)	28 (27-29.75)	.553
RAVLT Trial 1			
Mean (SD)	3.82 (1.54)	4.35 (1.73)	
Median (IQR)	4 (3-5)	4 (3-5)	.342
RAVLT Trial 5	8 (3.07)	9.63 (2.73)	.095
RAVLT Total	31.36 (9.85)	37.28 (10.52)	.101
RAVLT Trial 6	6.64 (2.46)	7.73 (3.40)	.328
RAVLT Delay	5.82 (3.34)	7.25 (3.5)	.231
RAVLT Recognition			
Mean (SD)	10.82 (3.71)	13.18 (2.49)	
Median (IQR)	10 (7-15)	14 (12.25-15)	.085
Rey Copying			
Mean (SD)	31.32 (8.10)	32.54 (6.27)	
Median (IQR)	34 (32-35)	35 (32-36)	.275
Rey Memory	12.32 (8.95)	18.7 (7.39)	**.019**
TMT A			
Mean (SD)	107.55 (117.1)	70.85 (48.04)	
Median (IQR)	63 (46-113)	57 (43.25-85.25)	.261
TMT B			
Mean (SD)	232 (205.77)	164.65 (90.26)	
Median (IQR)	98 (85-480)	142 (102.75-204.75)	.828
VFT Total	27 (10.33)	28 (10.42)	.779

MMSE – Mini Mental State Examination, RAVLT – Rey Auditory-Verbal Learning Test, TMT – Trail Making Test, VFT – Verbal Fluency Test, SD – Standard Deviation, IQR – Interquartile Range; Items described with both Mean and Median were analyzed with Mann-Whitney U; Items presented only with mean were analyzed using Student-T Test.

Impairment (MCI), signaling a high-risk of progression to dementia in the latter case [20]. However, the negative impact of ApoE4 on cognitive function is not inextricable from its association with AD; ApoE4 may also exacerbate the impact of other psychiatric and non-psychiatric illnesses on cognition, most notably depression [21] and multiple sclerosis [22].

There was also a noticeable trend towards poorer performance in verbal memory among ApoE4+ individuals, reflected particularly in the RAVLT Trial 5 and RAVLT Recognition tests. Considering its detrimental effects on memory and attention even in individuals without objective cognitive decline, it seems probable that a study with a larger number of participants could detect a significant difference between ApoE4+ and ApoE4- individuals. There is also data that is not entirely congruent with our previous conclusions, most notably a 2020 systematic review which concluded that ApoE4+ AD patients predominantly exhibit memory-related symptoms, while ApoE4- patients suffer greater impairments in executive function, language and visuospatial memory [6].

We hypothesize that the lower scores obtained by ApoE4+ individuals in cognitive tests are an early sign of neurodegenerative processes, which are not yet severe enough to register on the less sensitive MMSE test used for screening prior to inclusion in the study. This would be in line with the plethora of evidence that ApoE4 is associated with increased risk of developing AD, more severe disease phenotypes, and earlier onset of symptoms [4], [5]. The probable mechanism is that of ApoE4 precipitating "pathological" aging phenomena in the brain [23], resulting in a negative impact on cognition that may manifest as early as middle age. However, this is not to imply that all subjects with ApoE4 will invariably develop dementia, merely that they possess the most significant risk factor in the summation of factors that contribute to neurodegenerative disorders. In this sense, our study is a snapshot of a moment in time in which the pathological threshold has not yet been reached, but in which individuals that carry the ApoE4 gene variant are beginning to exhibit slight but quantifiable cognitive impairment.

The main limitation of our study is the small number of patients enrolled, possibly preventing us from adequately quantifying a number of smaller effects that ApoE4 may have on cognition. The principal strength of our study is that our two cohorts are perfectly matched by socioeconomic status, psychiatric and somatic comorbidities.

In conclusion, ApoE4 positive individuals performed slightly worse than controls on cognitive evaluations and the difference was most obvious regarding visual memory. It is possible that the effects of the ApoE4 genotype on cognition could be visible even in middle-aged persons, long before any significant changes would appear on a widely used cognitive test such as MMSE.

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