Title:

Is the apoE4 allele related to driving behavior in patients with amnestic Mild Cognitive Impairment (aMCI) and patients with Alzheimer's Disease (AD)?

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Σημείωμα του Συγγραφέα

Το δοκίμιο αυτό αποτελεί διπλωματική εργασία που συντάχθηκε για το Τμήμα Ιατρικής του Πανεπιστημίου Αθηνών και υποβλήθηκε τον Ιούλιο του 2018. Η συγγραφέας βεβαιώνει ότι το περιεχόμενο του παρόντος έργου είναι αποτέλεσμα προσωπικής εργασίας και ότι έχει γίνει η κατάλληλη αναφορά στην εργασία τρίτων, όπου κάτι τέτοιο ήταν απαραίτητο, σύμφωνα με τους κανόνες της ακαδημαϊκής δεοντολογίας

Abstract

Introduction: Although patients with AD and aMCI have driving difficulties, there is inconsistency in the literature for their severity. A factor that with deleterious effects on cognitive functions is Apoe e4.

Objective: The aim of the current study was to examine possible differences in the driving behavior between carriers and non-carriers of the apoe ε4 in the clinical stages of AD and aMCI. In addition, cognitive functions were explored between the two groups.

Methods: The sample constituted of 18 patients with aMCI and mild AD (M = 71.61 ± 9.25) carriers of the ε4 and N = 18 (M = 73.89 ± 8.10) non-carriers matched for clinical diagnosis with no significant differences in age, years of education, general cognitive ability, gender and driving experience. Patients undergone a thorough neurological and neuropsychological assessment and participated in a driving simulation experiment of a rural environment in low and high traffic volume conditions.

Results: In low traffic volume condition carriers of the apoe ε4 did not demonstrate any significant differences with non-carriers, while in high traffic volume condition carries of the apoe ε4 drove significantly slower and had lower variation in their speed from the heading vehicle than non-carriers. In addition apoe ε4 carriers indicated significantly worse consolidation process, as depicted by verbal episodic memory neuropsychological measures. Interestingly, only speed variation managed to surpass statistical control for multiple comparisons applied through Bonferroni corrections.

Conclusions: Apoe ε4 seems to be related to worse driving behavior at the clinical stages of AD and aMCI. Driving simulator seems to be a sensitive tool for the detection of even slight differences in cognitive and functional level, even within the clinical stages of aMCI and AD.

Key-words: apolipoprotein ε, driving behavior, Alzheimer's Disease, Mild Cognitive Impairment, cognitive functions, speed variation

Περίληψη

Παρότι οι ασθενείς με νόσο Alzheimer (ΝΑ) και οι ασθενείς με Ήπια Νοητική Έκπτωση (ΗΝΕ) παρουσιάζουν ελλείμματα στην οδηγική τους συμπεριφορά, στην βιβλιογραφία αποτυπώνεται διχογνωμία σχετικά με το εάν μπορούν τελικά να οδηγήσουν με ασφάλεια ένα όχημα. Το αλλήλιο της απολιποπρωτεΐνης ε4 (apoe ε4) αποτελεί σημαντικό επιβαρυντικό παράγοντα στις νοητικές λειτουργίες.

Στόχος: της παρούσας μελέτης ήταν η διερεύνηση πιθανών διαφορών στην οδηγική συμπεριφορά ασθενών με αμνησιακή ΗΝΕ (αΗΝΕ) και ασθενών με ήπια ΝΑ φορέων του apoe ε4 από αντίστοιχους ασθενείς που δεν το φέρουν στο γονότυπό τους.

Μέθοδος: Το δείγμα αποτελούνταν από 18 ασθενείς με αΗΝΕ και ήπια ΝΑ φορέων του apoe ε4 (M = 71.61 ± 9.25) και 18 αντίστοιχους ασθενείς (M = 73.89 ± 8.10) που δεν φέρουν το αλλήλιο ε4. Οι δύο ομάδες εξισώθηκαν ως προς την κλινική διάγνωση και δεν διέφεραν ως προς την ηλικία, το μορφωτικό επίπεδο και την οδηγική εμπειρία σε έτη, το φύλο, ούτε ως προς τη γενική νοητική κατάσταση. Εξετάστηκαν από ειδικό νευρολόγο και νευροψυχολόγο και συμμετείχαν σε πειραματική διαδικασία προσομοίωσης οδήγησης επαρχιακού περιβάλλοντος σε συνθήκες μειωμένου και αυξημένου κυκλοφοριακού φόρτου.

Αποτελέσματα: Από τη σύγκριση των δύο ομάδων προέκυψε ότι στη συνθήκη μειωμένου κυκλοφοριακού φόρτου οι φορείς του apoe ε4 δεν διαφέρουν από τους ασθενείς που δεν το φέρουν στο γονότυπό τους, ενώ στη συνθήκη αυξημένου κυκλοφοριακού φόρτου, οι φορείς του apoe ε4 παρουσίασαν στατιστικώς σημαντικά μικρότερη μέση ταχύτητα και μικρότερη διακύμανση ταχύτητας. Σε επίπεδο νοητικών λειτουργιών, η νευροψυχολογική εκτίμηση αποτύπωσε ισχυρότερα ελλείμματα στην λεκτική επεισοδιακή μνήμη στους φορείς του apoe ε4. Η εφαρμογή διορθώσεων με το στατιστικό κριτήριο Bonferroni, υπέδειξε ότι μόνο η διακύμανση τη ταχύτητας διαφέρει ανάμεσα στις δύο ομάδες.

Συμπέρασμα: Η οδηγική συμπεριφορά ασθενών με αΗΝΕ και ήπια ΝΑ φαίνεται να επηρεάζεται από την παρουσία του αλληλόμορφου ε4 στον γονότυπο των ασθενών. Η προσομοίωση οδήγησης αποτελεί ένα μέσω για την ανίχνευση αδρών διαφορών σε νοητικό και λειτουργικό επίπεδο ακόμη και σε κλινικά στάδια.

Λέξεις-κλειδιά: απολιποπρωτεΐνη ε, οδηγική συμπεριφορά, νόσος Alzheimer, Ήπια Νοητική Έκπτωση, νοητικές λειτουργίες, διακύμανση ταχύτητας

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Πρόλογος

Το έναυσμα για να ασχοληθώ με τις νοητικές και λειτουργικές μεταβολές –όπως αυτές αποτυπώνονται μέσω της προσομοίωσης οδήγησης- σε συνδυασμό το γονίδιο της απολιποπρωτεΐνης ε στο κλινικό πλαίσιο της Ήπιας Νοητικής Έκπτωσης και της νόσου Alzheimer, προήλθε από το ενδιαφέρον μου για τη σύνδεση νευροβιολογικών δεικτών με τον κλινικό φαινότυπο σε νευροεκφυλιστικές παθήσεις.

Η εκπόνηση της παρούσας εργασίας δεν θα είχε ολοκληρωθεί χωρίς την βοήθεια ενός συνόλου ανθρώπων. Πρωτίστως, θα ήθελα να ευχαριστήσω τον επόπτη μου κ. Σωκράτη Παπαγεωργίου, για την εμπιστοσύνη που μου έδειξε τόσο στο να αναλάβω μία διπλωματική εργασία με ισχυρό διεπιστημονικό χαρακτήρα όσο και σε όλη τη διάρκεια της συνεργασίας μας, για τις πολύτιμες παρατηρήσεις του και την ενθάρρυνση να «προχωράω» και να επιλύω πιθανές δυσκολίες που ανέκυπταν.

Επιπλέον, θα ήθελα να ευχαριστήσω την κα. Αλεξάνδρα Οικονόμου για τις πολύτιμες συμβουλές της σχετικά με την ερμηνεία των ευρημάτων και την παρατηρητικότητά της.

Επίσης, θα ήθελα να ευχαριστήσω τον Ίων Μπεράτη για όλο τον ποιοτικό χρόνο που διέθεσε στην διαχείριση μεθοδολογικών ζητημάτων και για τη γόνιμη συζήτηση που μας επέτρεψε να κατανοήσουμε την ουσία αυτής της μελέτης. Εξαιρετικά σημαντική ήταν επίσης η συμβολή της Διονυσίας Κονταξοπούλου και της Στέλλας Φραγκιαδάκη που σε όλη τη διάρκεια μου παρείχαν χρήσιμες συμβουλές και υποστήριξη, που συνέβαλαν καθοριστικά στην ολοκλήρωση αυτής της διπλωματικής εργασίας.

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Is the apoE4 allele related to driving behavior in patients with amnestic Mild Cognitive Impairment (aMCI) and patients with Alzheimer's Disease (AD)?

1. Introduction

1.1 Driving Behavior in Mild Cognitive Impairment and Alzheimer's Disease

According to recent epidemiological data from the European Union, it is 1.4 times more likely for the older people to get fatally injured in motor vehicle accidents. As the percentage of the advanced agers in society increases, the number of older licensed drivers is growing (Eurostats, 2014). Driving constitutes a multifactorial process combining demands on cognitive, sensory and physical functions (Anstey, Wood, Lord & Walker, 2005). According to Wagner, Müri, Nef and Mosimann (2011), visual attention, visual perception, executive functions, and memory are contributing to accomplish driving.

Alzheimer's Disease (henceforth AD) is the most prevalent neurodegenerative disorder and the main cause of major neurocognitive disorder worldwide, (WHO, 2015). A number of researchers demonstrate that AD adversely affects driving performance. During the mild stages of the disease patients with AD, although demonstrating driving errors, they seem to maintain their basic vehicular skills (Brown & Ott, 2004; Dawson et al., 2009; Carr, Duchek & Morris, 2000; Perkinson et al., 2005; Ernst et al., 2010). However, several studies highlight driving errors of patients with AD (Fitten, Perryman & Wilkinson, 1995; Bieliauskas, Roper, Trobe, Green & Lacy, 1998; Uc, Rizzo, Anderson, Shi & Dawson, 2006; Dawson, Anderson, Uc, Dastrup & Rizzo, 2009; Pavlou et al., 2016). Among the most critical driving performance deficits for patients with AD are low average speed (Fitten, 1995; Cox et al., 1998; Eby, Silverstein, Molnar, LeBlanc & Adler, 2012, Pavlou et al., 2015), low reaction time (Beliauskas et al. 1998; Fritteli Borghetti, Iudice, Bonanni, Maestri,

Tognoni, 2009; Vaux, Ni., Rizzo, Uc & Andersen, 2010) and high accident probability (Uc, Rizzo, Anderson, Shi & Dawson, 2004; Uc et al., 2006; Hunt, Morris, Edwards & Wilson, 1997; Ott et al., 2008; Rizzo, McGehee, Dawson, Andersen, 2001). In addition, some studies suggest that patients with AD have difficulty with the lateral position of the vehicle (Dawson et al. 2009; Fritteli et al., 2009; Cox et al. 1998), maintaining a proper headway distance (Pavlou et al., 2016), turning left (Uc et al. 2004; Cox et al., 1998) and disorientation issues with increased possibilities of getting lost (Uc et al., 2008; Eby et al., 2012).

Mild Cognitive Impairment (henceforth MCI) is a clinical diagnostic entity regarding 16-20% of the general population above the age of 65 (Roberts & Knopman, 2013), which is considered a preclinical stage of dementia –most commonly due to AD. Subjective concerns about deterioration of at least one cognitive domain, objective cognitive deficits for the individual's age, although with preserved functional activities and dementia excluded, constitute the clinical phenotype of MCI (Petersen & Morris, 2005). Memory complaints is the distinguishing feature among MCI phenotypes, enhancing the differentiation of amnestic from non-amnestic subtype (Petersen, 2004). Driving behavior of patients with MCI has also been investigated. Although MCI patients are generally considered safe drivers, according to some studies, suggesting that driving behavior of patients with MCI does not significantly deviate from the healthy elderly's (Devlin, McGillivray, Charlton, Lowndes & Etienne, 2012; Uc & Rizzo, 2008), there is a number of studies reporting important driving errors (Snellgrove, 2005; Bowers et al., 2013; Griffith et al., 2013). More specifically, seminal parameters for patients with MCI driving behavior are low average speed and increased reaction time (Pavlou et al, 2015). They, also, demonstrate difficulty in maintaining a proper lateral position of

the vehicle (Snellgrove, 2005; Wadley et al., 2009; Griffith et al., 2013) and a proper distance from the headway vehicle (Pavlou et al., 2015), as well as difficulty in left turns (Snellgrove, 2005; Wadley et al., 2009).

In accordance with the aforementioned, deciding whether a patient is able to continue driving is a complex procedure. Diagnosis of MCI or AD is insufficient to determine driving privileges withdrawal. Herein, a number of studies focus on personalized approaches when discussing on continuing or withdrawing driving (Papageorgiou et al., 2016; ManSon-Hing, Marshall, Molnar & Wilson, 2007, Brown & Ott, 2004).

1.2 Contributing factors in driving behavior

Various factors contribute to impaired driving behavior. According to Wagner et al., (2011), poor driving performance is a result of a combination including cause of dementia, disease stage, other comorbidities and compensation strategies that a patient might adapt. What is more, a recent meta-analysis by Hird, Egeto, Fischer, Naglie, & Schweizer (2016), concluded that severity of cognitive decline appears to have important predictive utility over driving ability in patients with AD and patients with MCI.

Among the factors that increase severity of cognitive defictis is the ε4 allele of the apolipoprotein ε (henceforth apoe ε4). Apoe ε4 is a well-documented genetic risk factor of AD (Harold et al., 2009) that increases the possibilities for amnestic-MCI (henceforth aMCI) in the healthy elderly (Michaud, Siahpush & Murman, 2017), and it accelerates the progression from MCI to dementia due to AD (Fleisher, Sowell, Taylor, Gamst, Petersen & Thal, 2007; Elias-Sonnenschein, Viechtbauer, Ramakers, Verhey & Visser, 2011). Patients with aMCI, carriers of the apoe ε4 progress with

increased rate to AD dementia than non-carriers (Farlow, He, Tekin, Xu, Lane, & Charles, 2004; Whitehair et al., 2010), while the severity of memory decline in MCI patients is highly associated with the apoe ε4 (Ramakers et al., 2008).

Cognitive deficits in apoe ε4 carriers are more severe in comparison with non-carriers, mainly affecting their memory. Patients with MCI positive to apoe ε4 have more distinct episodic memory deficits than non-carriers, while aMCI patients, carriers of the apoe ε4, have increased rate of deterioration in their cognition and everyday functionality than non-carriers (Farlow et al., 2004; Whitehair et al., 2010). Recent studies have depicted the episodic verbal memory deficits aMCI patients who are carriers of the Apoe ε4, (Kay et al., 2017) as well as visuospatial episodic memory impairment (Laczó et al., 2014). According to Smith et al. (1998), a group consisting of both patients with MCI and patients with AD positive to the ε4 allele has greater episodic memory deficits than non-carriers. In a recent study of Wang et al. (2015), AD patients, carriers of the ε4 allele, had worse performance in episodic memory and learning assessment. Interestingly, it has been suggested that the MCI apoe ε4 carriers have an overlapping cognitive profile with patients with mild AD (Farlow et al., 2004).

A recent study by Roe et al. (2017), underpinned a statistically significant associations of biomarkers which constitute pathological evidence of AD, namely higher tau and p-tau levels in comparison with amyloid beta in the cerebrospinal fluid and higher amyloid beta concentration in the brain based on PET-PiB, with driving errors in the healthy elderly. Although higher ratios of cerebrospinal fluid to amyloid beta and p-tau to amyloid beta and PIB Cortical Binding Potential were associated with a higher rate of driving errors, they were not with the neuropsychological measures. Taking into consideration the association between biomarkers of AD and

driving errors, even at an asymptomatic stage (Roe et al., 2017), we decided to examine whether the apoe ε4 allele affects driving performance in the clinical stages of aMCI and mild AD. To our knowledge, this is the first study comparing the driving behavior between apoe ε4 carriers and non-carriers.

1.3 Objectives

The aim of the current study was to examine whether the presence of the apoe ε4 in the genotype of patients with aMCI and mild AD affects their driving performance. Furthermore, by assessing cognitive functions with a thorough neuropsychological assessment we sought to explore a possible relationship between cognitive and driving measures. Our hypothesis was that carriers of the apoe ε4 would have worse driving behavior than non-carriers, even within the clinical stages of aMCI and AD, as well as greater episodic memory deficits.

Methods

1.4 Participants

In the current study, sample consisted of 18 carriers of the apoe ε4 (4 women and 14 men) and 18 non-carriers (3 women and 15 men). The participants were matched for the clinical diagnosis, as both groups contained 13 aMCI patients and 5 AD patients. Participants were referred to the Cognitive Disorders/Dementia Unit at the 2nd Department of Neurology at NKUA "Attikon" University General Hospital for evaluation. All the participants underwent a thorough neurological, neuropsychological and ophthalmological assessment. In order to participate in the current study, patients had to meet the following criteria: a) have a valid driving license, b) be active drivers: at least once a week, 10km per week and 2500km per year, c) be experienced drivers: having driven for more than three years, after acquiring their driving license, d) provide blood sample for apoe genotyping during their medical evaluation. Exclusion criteria contained: a) history of psychosis, b) significant motor or visual disorder, c) complains about dizziness or nausea while in a moving vehicle, d) record of traffic accidents regarding the last two years, e) evidence of alcohol or drug addiction.

2.2 Procedure

2.2.1 Medical/Neurological Evaluation

All participants undergone a comprehensive neurological and ophthalmological evaluation. A thorough medical history was reported for each patient that assessed memory complaints and their progression –verified by an informant, drug, family and social history. Motor ability, functionality of daily living

and neuropsychiatric symptoms were evaluated. For the exclusion of metabolic and structural causes of memory impairment, blood tests and brain MRI scans were evaluated. The diagnosis of aMCI was made according to Petersen and Morris criteria (2005) along with a score of 0.5 in the Clinical Dementia Rating Scale (CDR; Morris, 1993), while the diagnosis of AD was in accordance with the McKhann et al. criteria (2011) along with a score of 1 in the CDR. The diagnoses were all reached by the same behavioral neurologist (SGP) with expertise in cognitive disorders and dementia.

2.2.2 Neuropsychological Assessment

The cognitive functions were assessed with a thorough neuropsychological battery evaluating: a) general cognitive function (Mini Mental State Examination, MMSE, Folstein, Folstein & McHugh, 1975) b) executive functions (Frontal Assessment Battery, FAB, Dubois, Slachevsky, Litvan, & Pillon, 2000; Trail Making Test A and B, TMT A, TMT B, Reitan, 1979; Letter Number Sequencing, LNS, Wechsler, 1997; Symbol Digit Modalities written, SDMT, Smith, 1982; Semantic Verbal Fluency -category: animals), c) visuospatial perception and constructional ability (Judgement of Line Orientation, JLO, Benton, 1994; Clock Drawing Test, CDT, scored according to Freedman et al., 1994 criteria), d) verbal episodic memory (Hopkins Verbal Learning Test- Revised, HVLT-R, Benedict, Schretlen, Groninger & Brandt, 1998) e) visuospatial episodic memory (Brief Visuospatial Memory Test-Revised, BVMT-R, Benedict, Schrelten, Grononger, Dobraski, Shpritz, 1996).

2.2.3 Apoe genotyping

During the extensive medical evaluation, venous blood was drawn from each individual in order to extract their apolipoprotein ε genotype. Blood was collected in

tubes containing sodium ethylenediaminetetraacetic acid and stored frozen at -20 °C. Plasma samples were centrifuged at 15,000 rpm at 4 ° C for 15 minutes. DNA isolation was performed with the High Pure PCR Template Kit by Roche, followed by apoe genotyping with the real time Polymerase Chain Reaction (PCR) methodology and melting curve analysis method in the Light Cycler platform. In case of ambiguous results, PCR-RFLP was also performed as a confirmative test (Hixson and Vernier, 1990).

2.2.4 Driving Simulator Experiment

For the assessment of the driving behavior of the participants, a driving simulator experiment was conducted. The driving simulator is a Foerst FPF, composed by three LCD screens 40" (full HD: 1920x1080pixels), driving position and a support motion base. The dimensions of the overall construction are 230x180cm, the field of view is 170 degrees and the width of the base is 78cm. The simulator was validated against real word environment (Yannis, Papantoniou & Nikas, 2015; Vardaki, Dickerson, Beratis, Yannis & Papageorgiou, 2015).

The experimental procedure took place at the Department of Transportation Planning and Engineering of the National Technical University of Athens. The participants after a small practice session, drove in a simulated rural environment. The main characteristics of the rural road were: a) distance of 2.1km, b) speed limit 70km/h, c) a 3 meter wide single lane with zero gradient and mild curves. Two unexpected incidents occurred at fixed points on the roadway: a deer and a donkey appeared suddenly on the road. There were two conditions considering the traffic volume: a. low traffic volume Q = 300 vehicles per hour, b. high traffic volume Q = 600 vehicles per hour.

For the current study, the driving indexes that were examined based on the simulation experiment were: a) average speed (in km/h), b) the speed variation (standard deviation), c) distance from the heading vehicle (in meters), d) variation of the distance from the heading vehicle (standard deviation), e) lateral position (distance from the road axis in meters), f) variation of the lateral position (standard deviation) g) reaction time in unexpected incidences (in milliseconds), h) accident probability.

2.2.5 Ethical Considerations

This study is in accordance with the Helsinki Declaration and has been approved by the Research Ethics Committee of the Attikon General University Hospital. After providing the participants with written and oral information about the study, a written consent was signed. In the consent there was a request for an additional permission to conduct genetic evaluation related to neurodegenerative disorders. It was clarified to the patients that their participation would be voluntary and that they had the right to withdraw any time. It was highlighted that their personal data would remain confidential and would only be used for research purposes.

2.3 Statistical Analysis

For the analysis of the current study SPSS software was used. Statistical significance level was $p < .05$. Independent samples t-test were used for the comparison between carriers and non-carriers of the apoe ε4. However, for some of the driving and neuropsychological indexes examined, the normality hypothesis was violated. For the specific dependent variables, non-parametric Mann-Whitney U tests were also conducted. Parametric and non-parametric tests did not demonstrate any differentiation regarding the detection of statistically significant findings. In the

results section both parametric and non-parametric tests are reported. In addition, in order to explore the possible relationship between cognitive and driving indexes, Pearson r correlations were used. Finally, in order to control the familywise error rate, due to multiple comparisons, both in comparisons regarding neuropsychological and driving indexes and in correlations between them, Bonferroni corrections were applied. However, because of the fact that there in not yet a universally accepted approach on how to deal with the problem of multiple comparisons, the results reported in this section will be discussed both before and after the application of Bonferroni corrections.

2. **Results**

3.1 Demographics

A chi-square test of independence was performed to compare the frequency of each gender between apoe ε4 carriers and no-carriers. The two groups were similar in terms of gender, X^2 (1, N = 36) = .177, p = .674. Independent samples t-test were conducted in order to compare the two groups in age, education years, driving experience and general cognitive ability, as measured by the Mini Mental State Examination (MMSE, Folstein and Folstein, 1975) (Table 1). So, the two groups were similar in terms of their demographic characteristics. [Additionaly: Kolmogorov-Smirnov tests indicated that the assumption of normality was violated for the performance of carriers of the apoe ε4 in the MMSE score $D(9) = .28$, $p = .039$, so Mann Whitney U test was conducted, indicating that apoe ε4 carriers (Mdn = 26.00) did not differ significantly from the non-carriers (Mdn = 28.00), U = 132.00, p = .339.]

Table 1

Demographic characteristics of carriers and non-carriers of the Apoe ε4

	ApoE4 carriers (n =18)		ApoE4 non-carriers (n =18)			
Demographic Information	*M*	*SD*	*M*	*SD*	*t*	*p*
Age	71.61	9.25	73.89	8.10	.79	.438
Education years	11.78	3.90	11.56	4.69	-.15	.878
Driving Experience	42.92	11.69	45.73	8.57	.65	.521
MMSE score	25.78	5.16	25.61	3.31	-.12	.909

Note: *$p < .05$. **$p < .01$. ***$p < .001$.

3.2 Driving Behavior

In order to examine for possible differences between carriers and non-carriers in their driving performance t-tests of independent samples were used. First, the data

from the low traffic volume rural environment were analyzed, followed by the analysis for high traffic volume conditions in the rural area. As reported in Table 2, in low traffic volume conditions, there were no significant differences between carriers of the apoe ε4 and non-carriers. [Additionaly: In average reaction time, normality test with Kolmogorov-Smirnov indicated that performance of the carriers of the apoe ε4 violated the normality assumption $D(9) = .30$, $p = .018$, as a result the non-parametric Mann-Whitney U test was used indicating that carriers of the apoe ε4 (*Mdn* = 1800.50) did not have significant differences from non-carriers (*Mdn* = 2016.50), $U = 104.00$, $p = .724$. Regarding accident probability, Kolmogorov-Smirnov indicated both carriers of the apoe ε4 $D(9) = .40$, $p < .001$ and non-carriers $D(10) = .31$, $p = .003$ violated the normality assumption. Mann-Whitney U test did not demonstrate any differences between apoe ε4 carriers (*Mdn* = .00) and non-carriers (*Mdn* = .00), $U = 100.00$, $p = .501$.]

Independent samples t-tests for the driving performance in high traffic volume conditions, as presented in Table 2, indicated that patients with the apoe ε4 allele had significantly lower average speed, as well as speed variation, than non-carriers. However, the two groups did not have any other significant differences in their driving performance. [Additionally: In lateral position variation, Kolmogorov-Smirnov test depicted that performance of the carriers of the apoe ε4 violated the assumption of normality $D(9) = .34$, $p = .004$. Mann-Whitney U test did not indicate significant differences between apoe ε4 carriers (*Mdn* = .25) and non-carriers (*Mdn* = .25), $U = 115.00$, $p = .624$. Kolmogorov-Smirnov normality test indicated also that performance of the non-carriers violated the assumption in average reaction time $D(10) = .26$, $p = .046$. Mann-Whitney U test demonstrated that average reaction time of carriers of the apoe ε4 (*Mdn* = 2217.00), did not significantly differ from non-

carriers (*Mdn* =1995.75), $U = 103.50$, $p = .242$. In addition, in accident probability the performances of both carriers $D(9) = .26$, $p < .001$ and non-carriers were not normally distributed according to Kolmogorov-Smirnov test $D(10) = .42$, $p < .001$. Mann-Whitney U test demonstrated that apoe ε4 carriers (*Mdn* = .00) did not significantly differ from non-carriers (*Mdn* = .00), $U = 119.00$, $p = .362$ in accident probability.]

Bonferroni corrections were applied, in order to control for the familywise errors, demonstrating that only the speed variation in the high traffic volume conditions, differentiated carriers ($M = 7.74$) from non-carriers ($M = 11.17$) of the apoe ε4, $t(30) = 4.36$, $p < .001$, $d = .70$.

Table 2

Driving performance of ApoE4 carriers and non-carriers in low and high traffic volume conditions

	ApoE4 carriers (n = 18)		ApoE4 non-carriers (n = 18)				
Driving indexes	*M*	*SD*	*M*	*SD*	*t*	*p*	*d*
Low traffic							
Speed	36.59	7.35	39.62	6.32	1.19	.246	-
Speed SD	9.87	2.47	11.73	2.78	1.91	.066	-
Lateral Position	1.53	.15	1.49	.11	-.84	.407	-
Lateral Position SD	.28	.04	.29	.04	.81	.424	-
Heading	547.98	155.63	542.82	131.71	.10	.924	-
Heading SD	244.89	72.66	227.91	56.19	-.70	.490	-
Reaction Time	2083.80	757.51	1997.67	332.99	-.40	.690	-
Accident Probability	.27	.59	.33	.49	.34	.739	-
High traffic							
Speed	32.63	7.06	38.23	6.13	2.40	.023*	.85
Speed SD	7.74	1.50	11.17	2.77	4.36	.000**	.70
Lateral Position	1.64	.10	1.62	.12	-.55	.586	-
Lateral Position SD	.26	.04	.27	.05	.84	.407	-
Heading	401.63	214.10	302.31	106.47	-1.66	.107	-
Heading SD	204.75	80.39	157.37	52.14	-1.99	.057	-
Reaction Time	2438.35	705.964	2184.75	643.06	-1.08	.290	-
Accident Probability	.18	.53	.31	.60	.69	.495	-

Note: *$p < .05$. **$p < .01$. ***$p < .001$. Low traffic: low traffic volume condition, Speed: Average Speed, Speed SD: Speed Variation, Lateral position SD: Lateral Position Variation, Heading: Distance from Heading Vehicle, Heading SD: Distance from Heading Vehicle Variation, high traffic: high traffic volume condition.

3.3 Neuropsychological Measurements

Table 3

Performance of ApoE4 carriers and non-carriers in Neuropsychological measures

	ApoE4 carriers (n = 18)		ApoE4 non-carriers (n = 18)				
Cognitive Measures	*M*	*SD*	*M*	*SD*	*t*	*p*	*d*
Executive Functions							
FAB	12.89	2.83	12.56	3.38	-3.21	.750	-
Semantic Fluency	15.50	7.12	14.72	4.47	-.39	.697	-
Phonemic Fluency	10.83	3.63	8.78	3.59	-1.71	.097	-
TMT-A	72.39	58.27	70.83	26.60	-.10	.919	-
TMT-B	166.33	81.78	219.17	92.43	1.82	.078	-
LNS	6.11	3.46	6.22	3.39	.10	.923	-
SDMT	26.53	16.67	19.83	11.62	-1.39	.175	-
Episodic Memory Verbal							
HVLT-R total	14.33	6.3	15.44	4.90	.59	.556	-
HVLT-R-DR	2.83	5.35	2.00	2.50	-.60	.553	-
HVLT-R-REC	8.22	2.92	9.78	1.59	1.98	.055	-
HVLT-R-DI	5.39	3.26	7.67	2.64	2.31	.027*	.80
Episodic Memory Visuospatial							
BVMT-R total	11.33	7.28	9.44	7.77	-.75	.457	-
BVMT-R-DR	4.17	3.76	3.56	3.19	-.53	.602	-
BVMT-R-REC	4.83	1.25	5.33	.97	1.34	.189	-
BVMT-R-DI	4.06	1.51	4.17	1.89	.20	.847	-
Visuospatial Perception							
JLO	12.72	4.25	12.94	5.45	.14	.892	-
CDT	5.67	2.30	5.83	1.62	.25	.803	-

Note: $*p < .05$. $**p < .01$. $***p < .001$. FAB: Frontal Assessment Battery, TMT-A, TMT-B: Trails Making Test A and B, LNS: Letter Number Sequencing, SDMT: Symbol Digit Modalities Test, HVLT-R total: Hopkins Verbal Learning Test Total, HVLT-R DR: Hopkins Verbal Learning Test Delayed Recall, HVLT-R REC: Hopkins Verbal Learning Test Recognition, HVLT-R DI: Hopkins Verbal Learning Test Discrimination Index, BVMT-R total: Brief Visuospatial Memory Test Total, BVMT-R DR: Brief Visuospatial Memory Test Delayed Recall, BVMT-R REC: Brief Visuospatial Memory Test Recognition, BVMT-DI: Brief Visuospatial Memory Test Discrimination Index.

Independent samples t-test was conducted to compare the performance or carriers and non-carriers in neuropsychological measures. As presented in Table 3, non-carriers of the apoe ε4 outperformed carriers in verbal episodic memory measures, namely HVLT-R discrimination index. However, no other statistically significant discrepancies were depicted. [Additionaly: Kolmogorov-Smirnov

normality test indicated that the distribution in the performance in the CDT of the apoe ε4 carriers $D(9) = .37$, $p = .001$ and non-carriers $D(10) = .36$, $p = .001$ was not normal. Mann Whitney U test was used, indicating that apoe ε4 carriers' performance in CDT (*Mdn* = 7.00) was not significantly different from non-carriers'(*Mdn* = 6.00), $U = 145.50$, $p = .568$. What is more, Kolmogorov-Smirnov demonstrated violation of the assumption in the performance of apoe ε4 carriers in Phonemic Verbal Fluency $D(9) = .28$, $p = .044$, so a Mann Whitney U test was used, indicating no significant differences between apoe ε4 carriers (*Mdn* = 11.50), and non-carriers (*Mdn* = 8.00), $U = 105.50$, $p = .072$. Distribution of the performance of the apoe ε4 carriers in HVLT-R delayed recall task was also not normally distributed, according to Kolmogorov-Smirnov $D(9) = .31$, $p = .012$, so a Mann Whitney U test was used. There were no significant differences between apoe ε4 carriers (*Mdn* = .00) and non-carriers (*Mdn* = 1.00) in HVLT-R delayed recall task $U = 156.00$, $p = .839$. Furthermore, normality assumption was also violated in the performance of the apoe ε4 carriers $D(9) = .32$, $p = .009$ and non-carriers $D(10) = .48$, $p < .001$ in BVMT-R recognition. A Mann Whitney U test did not depict any significant differences between apoe ε4 carriers (*Mdn* = 5.00) and non-carriers (*Mdn* = 6.00) in BVMT-R recognition $U = 124.00$, $p = .196$. Apart from BVMT-R recognition, according to Kolmogorov-Smirnov test, neither the performance of the non-carriers in BVMT-R discrimination index was normally distributed $D(10) = .29$, $p = .018$. A Mann-Whitney U test was used, demonstrating that apoe ε4 carriers (*Mdn* = 4.00) did not differ significantly from non-carriers (*Mdn* = 5.00) in BVMT-R performance $U = 146.50$, $p = .616$. Finally, in TMT-A performance of the carriers of the apoe ε4 allele were not normally distributed, according to the Kolmogorov-Smirnov test $D(9) = .30$, $p = .017$, neither was the performance of non-carriers in TMT-B $D(10) = .31$, $p = .006$. So, Mann

Whitney U tests were conducted, indicating no significant differences between carriers of the apoe ε4 (*Mdn* = 49.00) and non-carriers (*Mdn* = 66.50), *U* = 123.50, *p* = .223 in TMT-A, neither between the performance of the apoe ε4 carriers (*Mdn* = 150.00) and non-carriers (*Mdn* = 229.50) in TMT-B *U* = 111.00, *p* = .102.]

Bonferroni corrections were applied, indicating that none of the neuropsychological measures reached statistical significance.

3.1 Relationship between driving indexes and neuropsychological measures

Pearson r correlations were conducted in order to explore the relationship between neuropsychological measures and driving indexes in which carriers of the apoe ε4 indicated significantly worse performance than non-carriers. As previously presented, the driving indexes in which non-carriers differed from apoe ε4 carriers are: a) average speed and b) speed variation from the high traffic volume condition, while only the second reached statistical significance when Bonferroni corrections were applied. Pearson r correlations were used for both carriers and non-carriers.

As presented in Table 4, for the patients who are apoe ε4 positive, average speed did not correlate significantly with any of the neuropsychological measures. Nevertheless, speed variation was correlated with SDMT which is an executive functions measure. Speed variability was also correlated with visuospatial episodic memory measurements: BVMT-R total, BVMT-R –Delayed Recall and BVMT-R Discrimination Index. Interestingly, no significant associations were demonstrated by the exploration of the relationship between cognitive tests and driving variables for the patients that their genotype was apoe ε4 negative.

Application of Bonferroni post hoc tests, in order to control for multiple comparisons effect indicated that the associations did not reach statistical significance.

Table 4

Pearson correlations between neuropsychological measures and driving variables in which Apoe ε4 carriers have worse performance than non-carriers

	ApoE4 carriers (n = 18)				ApoE4 non-carriers (n = 18)			
	Av.Speed		Speed Var.		Av.Speed		Speed Var.	
Cognitive Measures	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
General Cognitive Ability								
MMSE	.403	.122	.463	.071	.042	.878	.60	.824
Executive Functions								
FAB	.459	.074	.456	.076	.122	.651	.651	.570
Semantic fluency	.401	.124	.466	.069	.165	.542	.098	.717
Phonemic fluency	.167	.537	.168	.533	.084	.503	.069	.233
TMT-A’	-.063	.818	-.169	.532	-.273	-.273	-.169	-.169
TMT-B’	-.232	.387	-.493	.053	-.114	.675	.012	.965
LNS	.146	.590	.340	.198	.095	.727	.144	.595
SDMT	.130	.644	.611	.016*	.065	.812	-.033	.904
Episodic Memory Verbal								
HVLT-R total	.313	.237	.395	.130	-.244	.362	.009	.974
HVLT-R-DR	.245	.361	.223	.406	.020	.941	-.040	.882
HVLT-R-REC	.466	.069	.445	.084	-.050	.853	.057	.834
HVLT-R-DI	.073	.787	.298	.262	-.217	.419	-.145	.592
Episodic Memory Visuospatial								
BVMT-R total	.308	.246	.606	.013*	.095	.727	-.103	.703
BVMT-R-DR	.206	.445	.558	.025*	-.053	.845	-.158	.559
BVMT-R-REC	.460	.073	.463	.071	-.160	.554	-.272	.309
BVMT-R-DI	.308	.246	.555	.026*	-.292	.272	-.603	.014
Visuospatial Perception								
JLO	.153	.572	.150	.579	.095	.478	-.103	.789
CDT	.351	.182	.362	.169	-.053	.757	-.158	.798

Note: **p* < .05. ***p* < .01. ****p* < .001. MMSE: Mini Mental State Examination, FAB: Frontal Assessment Battery, TMT-A’, TMT-B’: Trails Making Test A’ and B, LNS: Letter Number Sequencing, SDMT: Symbol Digit Modalities Test, HVLT-R total: Hopkins Verbal Learning Test Total, HVLT-R DR: Hopkins Verbal Learning Test Delayed Recall, HVLT-R REC: Hopkins Verbal Learning Test Recognition, HVLT-R DI: Hopkins Verbal Learning Test Discrimination Index, BVMT-R total: Brief Visuospatial Memory Test Total, BVMT-R DR: Brief Visuospatial Memory Test Delayed Recall, BVMT-R REC: Brief Visuospatial Memory Test Recognition, BVMT-DI: Brief Visuospatial Memory Test Discrimination Index, Speed Var.: Speed Variation, Av. Speed: Average Speed, Heading SD: Distance from Heading Vehicle Variation.

3. **Discussion**

The objective of the current study was to evaluate driving behavior of patients with aMCI and mild AD, carriers of the apoe ε4, in comparison to non-carriers. Driving behavior was evaluated through a simulated rural environment in two conditions: low and high traffic volume. Driving parameters assessed were: average speed, speed variation, lateral position of the vehicle and its variation, the distance from the heading vehicle and its variation, the reaction time in unexpected incidences and accident probability. Another aspect of the current study was to examine possible discrepancies in cognitive deficits between the two groups, as those were demonstrated through a thorough neuropsychological assessment, in order to explore a possible relationship between cognitive and driving behavior deficits. The hypothesis of a possible effect of the apolipoprotein genotype -carriers of the apoe ε4 allele in comparison to non-carrriers- on the outcome driving and cognitive variables was partially confirmed. More specifically, carriers of the apoe ε4 had significantly lower speed and lower speed variation than non-carriers in high traffic volume conditions. Moreover, the effect of the apoe ε4 allele on episodic memory impairment was depicted. Interestingly, of all three cognitive and diving indexes aforementioned, only speed variation accomplished to reach statistical significance after the application of correction for multiple comparisons.

According to the results, patients with aMCI and mild AD that carry the apoe ε4 allele in comparison to the non-carriers did not have any significant differences in the low volume traffic condition of the simulated rural environment. Interestingly, when the traffic volume was doubled, significant differences were depicted. This result might be explained, under the notion that the high traffic volume condition places more functional and cognitive demands on the driver to cope with. Hence,

interaction with more stimuli and more decisions to be taken for the vehicular control, might challenge more their cognitive resources. This agrees with a recent study by Beratis et al., (2017), describing the effect of distraction in patients with MCI, which increases the cognitive demands during the driving process.

Furthermore, lower average speed and lower speed variability of the apoe ε4 carriers demonstrates a more conservative way of driving, which is compatible to the driving profile of patients with mild AD (Fitten, 1995; Cox et al., 1998; Eby et al., 2012; Pavlou et al., 2015) and patients with MCI (Pavlou et al., 2015), although only speed variation survived the control for multiple comparisons. Notwithstanding the impairments in driving behavior, driving indexes crucial for a safe operation of the vehicle, such as accident probability and reaction time in unexpected incidences, did not significantly differentiate the two groups. Notably though, driving simulation was sensitive enough for the detection of discrepancies possibly related to the apolipoprotein genotype of the patients, even after controlling for the familywise error, through the application of Bonferroni corrections.

In line with the second hypothesis of the current study, investigation of the cognitive impairments between the two groups indicated that non-carriers outperformed the carriers of the apoe ε4 in a verbal episodic memory measurement, namely the HVLT-R discrimination index. Discrimination index is a measure to evaluate the individual's ability to correctly recognize the previously studied information and is calculated by subtracting the false positives from the true positive answers. The psychometric capacity of the discrimination index is enhanced when examined together with the delayed recall performance, in order to reach an accurate neuropsychological interpretation regarding information retrieval (Lezak et al., 2012). Apart from the recognition discrepancy, apoe ε4 carriers and non-carriers had equally

impaired performance in the delayed recall task of the HVLT-R. Hence, this pattern of findings implies a more severely impaired consolidation process in the apoe ε4 carriers. It should be mentioned that this difference did not survive statistically after the application of the Bonferroni corrections. This is in accordance with the idea suggested by O'Donoghue, Murphy, Nobre and Mackay (2018) -although for cognitively healthy elderly, implying that small samples could be sufficient to detect subtle differences, provided that sensitive neuropsychological measures are selected for cognitive domains affected by apoe ε4 allele. This was not the case in the current study, in which the number of the explored cognitive functions was disproportionally big for the relatively small sample size, due to the study's exploratory orientation, regarding the possible cognitive discrepancies between carriers and non-carriers of the apoe ε4 within symptomatic stages of aMCI and AD. However, through a directional hypothesis on the deleterious effects of the apoe ε4 specifically on episodic memory, this comparison might had reached statistical significance.

Apoe ε4 carriers worse performance in verbal episodic memory is in accordance to previous studies, assessing the cognitive functions based on the apolipoprotein genotype of the patients (Farlow et al., 2004; Whitehair et al., 2010; Kay et al., 2017; to Smith et al., 1998; Wang et al., 2015). A possible explanation for this result might be that the sample consisted of patients within the clinical stages of both aMCI and AD, so a considerable percentage of them had hippocampal atrophy, due to neurodegeneration. Apart from that, apoe ε4 has been associated with lower medial temporal lobe volume in AD and MCI (Geroldi et al., 1999; Dhikav & Anand, 2011; Shi et al., 2014). Herein, in terms of structural neuroanatomy, patients who carry the apoe ε4 combined the hippocampal atrophy related to the clinical diagnoses with the possible effects of the e4 allele in the medial temporal lobe volume. So the

anatomical implications could explain the apoe ε4 carriers' greater deterioration in consolidation in comparison to non-carriers, who, despite the impaired recall of information, manage to maintain their recognition performance in a better condition. It should be highlighted, though, that analyses of the brain MRIs of the patients was not in the objectives of the current study, so the aforementioned explanation can only be theoretically supported.

Although the rationale of the current study was that apoe ε4 allele might worsen driving behavior because of its effect on cognitive functions, this was not confirmed. More specifically, investigation of possible associations between driving and cognitive indexes, indicated that only speed variation of apoe ε4 carriers in the high traffic conditions of the rural environment correlated with neuropsychological indexes regarding visuospatial episodic memory and executive functions. However, after the application of Bonferroni corrections, these correlations lost their statistical significance. Apart from that, considering the cognitive consequences of the apoe ε4 occurrence in the phenotype, in order to support a possible mediatory role between cognitive and driving indexes, the cognitive function of interest would be verbal episodic memory, because of the observed differences of the apoe e4 carriers and non-carriers in the current study. Nonetheless, tasks of verbal episodic memory, and HVLT-R discrimination index –to be more precise, did not indicate any significant associations with the examined driving variables. A possible explanation might be reflected on the fact that verbal episodic memory is not a seminal cognitive function for driving behavior in mild stages of AD neither MCI, although in moderate and severe stages of AD it has a contributing role (Wagner et al., 2011). Notably, non-carriers did not have any associations between driving and cognitive measures, even before the application of Bonferroni corrections.

To our knowledge, this is the first study to compare driving behavior based on the apolipoprotein genotype of patients within the symptomatic stages of aMCI and mild AD. As previously mentioned, a recent study by Roe et al. (2017) explored AD biomarkers in preclinical asymptomatic stages of healthy ageing with driving errors, coming across strong correlations between driving errors and AD biomarkers, demonstrating their predictive utility on functional outcomes. Nevertheless, no association between neuropsychological measures with driving errors was indicated. The researchers interpreted this result suggesting that AD biomarkers only slightly affect cognitive functions and functionality in preclinical stages. However, in complex procedures as driving, these slight deficits are unraveled. In the same vein, the present study, despite the lack of statistically significant associations between driving and cognitive indexes, accomplished to describe impaired driving behavior, namely lower average speed and lower speed variation, even in genetic level using a driving simulator. Interestingly, only the difference on speed variation between carriers and non-carriers of the apoe ε4, which is a driving index, accomplished to surpass the control for familywise errors, but the neuropsychological measure did not. These results imply that the driving simulator is a tool sensitive to subtle functional changes and in combination with the neuropsychological assessment it has the capacity to provide a more thorough picture of the driver with neurodegenerative disease leading towards a more personalized and holistic approach, as latest literature suggests (Papageorgiou et al., 2016; ManSon-Hing, et al., 2007; Brown & Ott, 2004).

In the present study, there were limitations that should to be reported. Firstly, driving behavior was evaluated in a driving simulation environment. Although the

simulation was validated against real world conditions, on-road driving constitutes the gold standard for the driving behavior evaluation. Hence, future studies should consider an on-road driving evaluation when investigating biomarkers, as Roe et al. (2017) did, so as to accomplish better comprehension of the functional outcome. Another constraint of the current study was the relatively small sample size. As previously mentioned, a larger sample size might had provided a more accurate representation of the driving behavior and cognitive discrepancies between carriers and non-carriers of the apoe ε4 allele, considering that only large effect sizes were detected, as well as regarding the possible relationships between their driving performance and neuropsychological measures. It should be mentioned that, when exploring the possible effect of a genotype on the clinical phenotype –although in the current study phenotype is possibly more representative of the aMCI and mild AD neuropathology, sample size is a factor with important implications (O'Donoghue et al., 2018). Nonetheless, even if the sample was relatively small, it was large enough to detect the possible impact of the apoe ε4 on driving behavior and cognitive functions, in two groups matched for the clinical diagnosis and similar in terms of age, education years, driving experience and general cognitive ability. Due to the small number of participants, aMCI and mild AD were evaluated together in the current study, so heterogeneity of the diagnoses, despite matched between the two groups, might merely affected the clinical phenotype within each group.

In conclusion, the identification of sensitive tools which assess complex procedures such as the driving behavior, makes feasible the exploration of even slight cognitive and functional differences related to genetic biomarkers even within the clinical stages of aMCI and mild AD. Future studies, should explore the possible effect of the apoe ε4 in preclinical stages of AD, where the differences between

carriers and non-carriers would be even more subtle, in order to increase the current insight regarding the sensitivity of a driving simulation in detecting and evaluating functional and cognitive features.

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