**Journal of Genetics** (2022)101:41
https://doi.org/10.1007/s12041-022-01384-9



**RESEARCH ARTICLE**

# Missing apolipoprotein E ε4 allele associated with nonamnestic Alzheimer's disease in a Tunisian population

SALOUA FRAY[1,3]*, AFEF ACHOURI-RASSAS[1,2,3], SAMIR BELAL[3] and TAIEB MESSAOUD[2]

[1]*Department of Neurology, Charles Nicolle Hospital, Tunis, Tunisia*
[2]*Biochemistry and Molecular Biology Laboratory, Children's Hospital, Bab Saadoun, 1029 Tunis, Tunisia*
[3]*Faculty of Medicine of Tunis, Tunis El Manar University, 15 Rue Djebel Lakhdhar, La Rabta., 1007 Tunis, Tunisia*
*For correspondence. E-mail: fray_saloua@yahoo.fr, fraysaloua82@gmail.com.

Received 25 August 2021; revised 19 June 2022; accepted 14 July 2022

**Abstract.** In this study, we investigate the impact of apolipoprotein E epsilon 4 (APOE ε4) as a major risk factor of Alzheimer's disease (AD), based on the clinical presentation of the disease in our population on the one hand, and comparison of the results with the findings from the literature on the other hand. Our study covered a population of 144 patients versus 90 healthy controls matched with each other in terms of age, gender, age of onset, etc. All patients underwent neurological examination, comprehensive neuropsychological assessment and brain magnetic resonance imaging. Controls were selected based on the neurological examination and the Arabic version of the mini-mental state examination (MMSE). Patients were classified as probable typical amnestic AD and atypical nonamnestic AD if the patient had logopenic variant primary aphasia, posterior cortical atrophy, behavioural or dysexecutive variants, corticobasal syndrome, nonfluent and semantic variants of primary progressive aphasia associated to biological diagnosis for AB42, Tau and Ptau biomarks in the cerebrospinal fluid. Genotyping was performed using the polymerace chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The study of the allelic frequency of APOE in cases and controls show that APOE ε4 is associated with an increased risk for AD ($P = 0.002$). We observed that the distribution of APOE ε4 within the AD group differs depending on the phenotype. Nonamnestic AD was more common in patients not carrying APOE ε4 (APOE ε4 (−)) compared to carriers of homozygous or heterozygous APOE ε4 (APOE ε4 (+)) ($P = 0.038$). In addition to its known effect as a major risk factor, we found that patients with AD are APOE ε4 negative, they show cognitive decline in nonmemory domains (language, behaviour, attention, executive and visuospatial functions).

**Keywords.** Alzheimer's disease; genetic analysis; apolipoprotein E; Tunisian population; neurodegenerative disease.

## Introduction

Alzheimer's disease (AD) is the most common form of dementia. It is a chronic progressive neurodegenerative disease characterized by memory loss and deficits in other cognitive abilities, among which typically the impairment of anterograde episodic memory. A small number of patients show focal cortical symptoms, such as impaired visuospatial function, language, or executive function, while memory remains preserved for some time (van der Flier *et al.* 2011; Warren *et al.* 2012). This nonmemory phenotype is seen in roughly a quarter of patients (Snowden *et al.* 2007). Evidence from a twin study showed that the heritability for AD was 60–80% (Gatz *et al.* 2006). Most cases of early-onset AD are caused by mutations in the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) genes (Goate *et al.* 1991; Sherrington *et al.* 1995; Levy-Lahad *et al.* 1995). Genomewide association studies (GWAS) identified susceptibility loci for late-onset AD. Among them, the *APOE* gene is the strongest risk factor for late-onset AD. The *APOE* gene is located on chromosome 19q13.2 with a total of 3597 bases with four exons and three introns, and the gene has three major alleles (″2, ″3, and ″4). Apolipoprotein E (APOE), a lipid transport protein in plasma and central nervous system, is the major risk factor for AD which has been shown to modify the clinical presentation and rate of cognitive decline in AD (van der Flier *et al.* 2011). Human APOE isoforms modulate AD pathogenesis primarily through their differential effects on Aβ clearance and aggregation but they affect multiple pathways that are not necessarily dependent on Aβ. The mechanism can be a gain of toxic function or a loss of physiological

Published online: 06 September 2022

function (Yamazaki *et al*. 2019). It puts forward the hypothesis that *APOE* ε4 allele status is a driving factor for differences in the manifestations of the disease and the conflicting results regarding the influence of *APOE* ε4 which remains unclear (Smits *et al*. 2015). Thirty-eight per cent of patients with AD pathology under the age of 60 (Balasa *et al*. 2011), and 25% of all AD patients (Whitwell *et al*. 2012), do not complain of early memory loss, but instead express other cognitive complaints and can be referred to as atypical presentations of AD. Less is known about the role of the *APOE* genotype in patients with atypical AD. Studies have found varying results concerning the *APOE* ε4 allele frequency in atypical AD. Some researchers have found that the frequency of the *APOE* ε4 allele is lower in logopenic progressive aphasia and posterior cortical atrophy compared to typical AD (Mesulam *et al*. 1997; Schott *et al*. 2006; van der Flier *et al*. 2006; Rogalski *et al*. 2011; Josephs *et al*. 2014; Phillips *et al*. 2019) although others have observed similar frequencies, particularly between posterior cortical atrophy and typical Alzheimer's dementia (Tang-Wai *et al*. 2004; Lehmann *et al*. 2013; Carrasquillo *et al*. 2014).

We aimed to verify whether *APOE* ε4 is a major risk factor for AD in our Tunisian population as it was proven in previous studies (Rassas *et al*. 2012) and to investigate AD phenotype (amnestic versus nonamnestic AD) depending on APOE ε4 status, especially since only a few studies examined the impact of APOE genotype in north African mediterranean Arab AD patients.

## Materials and methods

### *Subjects*

Our study made use of the data collected during the period 2014–2016 at the memory consultation of the neurology department which involved 234 subjects: 144 AD patients and 90 healthy controls matched for age and education to the group. Using structured interview of the patient and caregiver, we reviewed relevant aspects such past medical history, age of onset, initial clinical expression, etc. All patients underwent neurological examination and at least two annual comprehensive neuropsychological assessments. All patients underwent brain magnetic resonance imaging (MRI), and laboratory blood tests to rule out nondegenerative causes of cognitive impairment. Structural brain MRI with cerebral coronal section T1 was performed to rule out others diagnosis of dementia, to study qualitative hippocampus atrophy according to the scale of Scheltens and to show other lobar atrophy. Reliable and standardized neuropsychological tests in conformity with the local cultural standards and normative data scores were used. This included the Mini Mental State Examination (MMSE), the Alzheimer's disease Assessment Scale Cognitive subscale, Frontal Assessment Battery, Geriatric Depression Scale, Instrumental Activities of Daily Living scale, and the Clinical Dementia Rating (Bellaj *et al*. 2008; Attia Romdhane *et al*. 2008). The neuropsychological assessment showed affected cognitive domains like attentionnel process, episodic memory, executive function, visuospatial function, praxis, gnosis, language and behavioural disorders. At the time of their inclusion in the study, all patients had already cognitive disorder with deficits involving memory and other cognitive abilities notwithstanding their amnestic or nonamnestic phenotype. Patients were classified according to initial clinical presentation and evolution of neuropsychological assessment in typical amnestic AD form and atypical nonamnestic form. Dementia diagnosis was established by a team of trained neurologists and neuropsychologists using Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) criteria (American Psychiatric 2013) in amnestic AD group (amnestic syndrome of hippocampal type) and clinically associated with CSF biomarkers in the nonamnestic AD group. The nonamnestic AD group is defined by clinicobiological criteria. We retained nonamnestic AD or atypical AD presentation when the initial neurocognitive impairment was primary progressive aphasia variants (leugopenic, nonfluent and semantic), posterior cortical atrophy, psychiatric and behavioural (frontal AD) or dysexecutive variants and corticobasal syndrome (Dubois *et al*. 2021). Although episodic memory loss was present, it was not the initial and the most prominent sign. Over a year of clinical and psychologic follow-up period, patients with a nonamnestic clinical expression did not fulfill diagnostic criteria for another neurocognitive disorder. All patients underwent neurological exam and numerous neuropsychological evaluations. Further, the biological diagnosis for AB42 and Tau biomarkers in the cerebrospinal fluid (CSF) shows decrease in Aβ 42 levels below 500 pg/mL and increase in tau levels above 350 pg/mL in nonamnestic AD patients. Control group members have been recruited from primary care centres. They have been subjected to an anamnestic survey including personal and family history, a neurological examination, and a neuropsychological assessment based on the MMSE (Bellaj *et al*. 2008), and only those subjects with a normal neurological examination and an MMSE score above 26 points, and no personal or familial psychiatric or cognitive impairment, and alcohol or drug abuse history have been considered as normal subjects and included in the study. All subjects or their custodians provided informed consent to participate in the study. The research protocol was approved by the local Research Ethics Committee

### *Biological analysis*

A 5-mL sample of peripheral blood was collected and DNA was extracted using the salting out procedure (Miller *et al*. 1988). *APOE* was genotyped as described (Hixson and Vernier 1990). CSF was collected by standardized lumbar puncture in plastic (polypropylene) tubes. The CSF samples were centrifuged at 3000 *g* at 4°C for 10 min and

**Table 1.** Baseline characteristics of the study population.

| | Patients with AD (*n*=144) | Controls (*n*=90) |
|---|---|---|
| Mean of age (years) | 70.14 (±10.44) | 69.13 (±14.56) |
| Mean age at onset (years) | 66.79 (±9.04) | |
| Late onset AD (>65 years) | 70.8% | – |
| Early onset AD (<65 years) | 29.2% | – |
| Sex ratio | 0.71 | 0.45 |
| Mean MMSE | 19.16 (±8.08) | – |
| Typical AD | 68.5% | – |
| Atypical AD | 31.5% | – |

**Table 2.** Neuropsychological profil of patients with amnestic and nonamnestic AD.

| | Amnestic AD % (*n*=99) | Nonamnestic AD % (*n*=45) |
|---|---|---|
| Altered attentional process (%) | 68% | 50% |
| Episodic memory dysfunction | 100% | 80% |
| Dysexecutive syndrome | 60% | 70% |
| Language disordrers | 60% | 70% |
| Altered praxis | 60% | 70% |
| Altered gnosis | 20% | 40% |
| Depression | 45% | 40% |
| Behavioural disorders | 30% | 70% |

immediately deep frozen and stored at –80°C pending biochemical analyses. CSF Aβ42 and t-Tau were measured separately by commercially available ELISA kits (Invitrogen, USA).

### *Statistical analysis*

Data were entered and analysed using Statistical Package for Social Sciences IBM-SPSS 20. The Student *t*-test was used for continuous variables, allele and genotype distributions in patients and controls, which were compared using $\chi^2$ test. A *P* value less than 0.05 was considered statistically significant.

This work is part of research work on the genetics of Alzheimer's disease, which has received the approval of the local ethics committee at Bechir Hamza Children's Hospital (Tunis, Tunisia) (approval number 10/2021).

## Results

The assessment involved 144 AD patients and 90 controls. The mean age was 69.13 (±14.56) years for the normal controls and 70.14 (±10.44) years for the AD group. The gender distribution found a sex ratio of 0.71 for AD patients and 0.45 for controls. There was no statistically significant difference in age and gender between the two groups. The mean MMSE was 19.16 (±8.08) in the AD group (table 1). The affected cognitive domains in both amnestic and nonamnestic groups are shown in table 2. The neuropsychological assessment of different cognitive functions showed episodic memory dysfunction in both groups with a higher frequency in amnestic AD group. Patients with nonamnestic AD had more disorders in executive functions (language, gnosis and behaviour) than patients with amnestic AD. Structural brain MRI evaluated hippocampus atrophy by Scheleten score (when score was greater than or equal to 1 interpreted by radiologist taking age into account) showed atrophy in 58% of patients with amnestic AD, as opposed to 95% of nonamnestic AD who had no hippocampus atrophy ($P = 0.000$). CSF biomarkers was performed in nonamnestic AD patients. The mean Aβ42 was 349.53 ± 170.51 pg/mL, the mean Tau was 421,62 ± 99, 06 pg/mL.

APOE ε3/ε3 is the most common genotype in both groups with a higher frequency in controls (71.1% versus 54.2%). The genotypic distribution table shows that APOE ε4/ε4 is more frequent in AD group (11.1% ($n$ = 16)) than in controls, three of which only carried this genotype. The comparison of the six *APOE* genotype frequencies among patients and control groups showed a significant difference ($P = 0.038$) (table 3). The study of the allelic frequency of APOE in cases and controls shows that *APOE* ε4 is associated with an increased risk for AD and the association is statistically significant ($P = 0.002$) (table 3). With respect to *APOE* status, 80% of amnestic AD patients carry *APOE* ε4, while 61.8% do not have *APOE* ε4. No difference was observed in mean age, gender and mean MMSE between the two subgroups. In addition to the known effect of *APOE* ε4, we observed that the distribution of *APOE* ε4 within the AD group differs depending on the phenotype. Nonamnestic AD was more common in patients not carrying *APOE* ε4 (APOE ε4 (−) negative) (38.2%) compared to carriers of homozygous or heterozygous *APOE* ε4 (APOE ε4 (+) positive) (20%). This association is statistically significant ($P$=0.038) (table 4).

## Discussion and conclusion

Our study confirms the already established role of *APOE* ε4 as a risk factor for AD both in literature and in Tunisian population (Rassas *et al*. 2012). Our results showed a significant correlation between ε4 allele frequency and probable AD. The allelic frequency ε4 was higher in the AD group (25.3%) compared to the controls group (11.7%), this association is in favour of a significant correlation with *P* value at 0.002 ($P = 0.002$). The association of the absence of APOE4 with the nonamnestic form is the main finding of our study. Among patients in the AD group ($n = 144$), 68.5%

**Table 3.** Comparison of the frequency distribution of *APOE* genotypes and alleles in the cases and control groups.

| | E3/E3 | E3/E4 | E3/E2 | E4/E4 | E4/E2 | E2/E2 | *P* value | E3 | E4 | E2 | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls | 64 | 14 | 7 | 3 | 1 | 1 | $\chi^2 = 11.76$ | 149 | 21 | 10 | $\chi^2 = 13$ |
| | 71,10% | 15,60% | 7,80% | 3,30% | 1,10% | 1,10% | $P = 0.038$ | 82,80% | 11,70% | 5,60% | $P = 0.002$ |
| Patients | 78 | 38 | 9 | 16 | 3 | 0 | | 203 | 73 | 12 | |
| | 54,20% | 26,40% | 6,20% | 11,10% | 2,10% | 0,00% | | 70,50% | 25,30% | 4,20% | |
| Odds ratio | – | 2.22 | 1.05 | 4.37 | 2.46 | 0.00 | | | | | |
| IC | – | 1.11–4.46 | 0.37–2.98 | 1.22–15.68 | 0.25–24.23 | – | | | | | |
| *P* | – | **0.024** | 0.92 | **0.023** | 0.44 | 1 | | | | | |

$P < 0.05$ is significant. In this table E3/E4 and E4/E4 genotypes constitute risk factor for AD.

**Table 4.** Comparison of *APOE* genotypes in amnestic and nonamnestic AD.

| | Amnestic AD (68.5%) | Nonamnestic AD (31.5%) | *P* value | Odds ratio | IC | *P* |
|---|---|---|---|---|---|---|
| APOE 4 − | 61,80% | 38,20% | ***P* = 0.038** | 0.404 | 0.162–1.01 | **0.052** |
| APOE 4 + | 80% | 20% | $\chi^2 = 3.88$ | | | |
| E2/E3 | 16,70% | 83,30% | | | | |
| E2/E4 | 100% | 0% | | | | |
| E3/E3 | 66,10% | 33,90% | | | | |
| E3/E4 | 84,60% | 15,40% | | | | |
| E4/E4 | 67, 3% | 32,70% | | | | |

$P < 0.05$ is significant. In this table the difference in the two AD groups is significant with E4 genotypes carriers and noncarriers.

had an amnestic form and 31.5% had a nonamnestic form. Patients who did not carry *APOE* ε4 developed nonamnestic AD in 38.2% of cases. Our results showed the correlation of this phenotype (non-memory AD) with the absence of the ε4 allele ($P = 0.038$). Whereas those carrying one or two ε4 alleles presented typical AD (memory form) in 80% of the cases. Apolipoprotein E ε4 gene as a risk factor for AD has been shown to modify clinical presentation (van der Flier *et al*. 2011). However, its influence is less clear. The study of the effect of *APOE* on global cognitive disorders has ended up in conflicting results according to some studies (Kleiman *et al*. 2006; Tschanz *et al*. 2011). Our study aimed at analyse the influence of *APOE* status on cognitive domains other than memory in a Tunisian sample population with AD. We found that when AD patients are APOE ε4 negative, they show cognitive decline in nonmemory domains (language, behaviour, attention, executive and visuospatial functions). Smits *et al*. (2015) found that *APOE* ε4 negative patients decline faster than APOE ε4 positive in the language. Recently Whitwell *et al*. (2021) found that typical AD showed higher APOE ε4 frequencies than atypical AD only aged between 57 and 69 years (Whitwell *et al*. 2021). Our results partly agree with those studies compared to the correlation of the absence of *APOE* ε4 with impairment of cognitive domains other than memory. This finding is in line with the observation that *APOE* ε4 negative patients are associated with a nonmemory profile in an AD Tunisian sample population.

As concerns, the relationship between *APOE* and amyloid pathways, pathological studies of post-mortem brain tissue from AD patients have found that *APOE* ε4 exacerbates the intraneuronal accumulation of Aβ (Christensen *et al*. 2010), plaque deposition in the brain parenchyma (Tiraboschi *et al*. 2004; Kok *et al*. 2009), formation of neurotoxic Aβ oligomers and the severity of cerebral amyloid angiopathy (Rannikmäe *et al*. 2014; Shinohara *et al*. 2016). Imaging studies have shown that *APOE*ε4 is consistently associated with greater Aβ deposition in the brains. A longitudinal study also showed that *APOE*ε4 carriers present increased Aβ deposition in the cortex compared with *APOE*ε4 noncarriers (Mishra *et al*. 2018). Some studies evoke that genetic profile leads to the distribution of pathology and suggest a region-specific biological effect of the ε4 allele in the brains of AD patients; it has been shown that *APOE* ε4 negative patients have more frontal and parietal atrophy, while *APOE* ε4 positive patients had more temporal and hippocampal atrophy (Geroldi *et al*. 1999; Pievani *et al*. 2009). Further, previous studies have shown that *APOE* ε4 negative patients with early-onset AD are at high risk of developing global brain atrophy compared to *APOE* ε4 positive patients with late-onset AD who had more pronounced hippocampal atrophy (Sluimer *et al*. 2008). Using Pittsburg compound B and fluorodeoxyglucose PET scans, Ossenkoppele *et al*. (2013) found a reversed *APOE* ε4 dose effect amyloid deposition in the frontal lobe, whereas *APOE* ε4 carriers' hip was associated with more profound

metabolic impairment in posterior parts of the cortex (Ossenkoppele *et al*. 2013). Many studies showed that the presence of more *APOE* ε4 alleles results in increased hippocampal atrophies on AD (Saeed *et al*. 2018). Recently, Foo *et al*. (2021) showed associations between AD polygenic risk scores, constructed using genomewide significant AD risk variants, and hippocampal subfield volumes and that this association was driven by the single-nucleotide polymorphisms (SNPs) in the *APOE* locus (Foo *et al*. 2021). Others found that the rate of decline of hippocampal parenchymal fraction in *APOE* ε4 carriers was significantly faster than in noncarriers (Ardekani *et al*. 2020). The Papez circuit was first described as the anatomic basis of emotion. Subsequent studies consolidated recognition of its limbic activities but showed a more important role in memory. Much attention has been devoted to characterize *APOE* ε4 related changes in the hippocampus, but AD pathology is known to spread through the whole of the Papez circuit. Other studies demonstrate changes in the structural association between hippocampal formation and thalamo–striatal connections occurring in mild cognitive impairment APOE ε4 carriers. These findings suggest that the ε4 allele may affect specific regional atrophy process (thalamus, hyppocampus) and that both regions had a strong structural covariance association with the left caudate nucleus. Also there is a dose-dependent effect of the *APOE* genotype on the regional brain atrophy (Novellino *et al*. 2019). Despite its very important role as a major risk factor for Alzheimer's disease, all AD patients do not carry *APOE* ε4. This suggests that there exist other environmental and genetic factors involved in the genesis of AD which could explain the phenotypic variations. Fan *et al.* (2019) focus on the influence of genetic factors, including the *APOE* gene, the interaction between *APOE* and other genes, and the polygenic risk factors for cognitive function and dementia. They also describe the effects of the associations between *APOE* and other genetic risk factors on cognition and the brain that exhibit a complex gene–gene interaction, and consider the importance of using a polygenic risk score to investigate the association between genetic variance and phenotype (Fan *et al*. 2019). Although the *APOE* gene explains partly the genetic risks associated with AD, other genes may still modify the *APOE* ε4 effect (Martínez *et al*. 2009).

The association between genetic predisposition and neuropsychological phenotype highlighting APOE's involvement in AD cognitive profile is among the strengths of our study. All the more so it covered an ethnic group that was not sufficiently studied, i.e. North African Mediterranean Arab population. But on the other hand, our study remains limited due to the small size of our population and the lack of correlation between APOE and other parameters involved in the pathophysiology of AD, particularly the levels of A-beta and tau. Going deeper in the study of this association and enlarging the size of the study population shall constitute an indisputable opportunity for better understanding and explaining the physiopathology of AD. In conclusion, we confirm that *APOE* ε4 is a major risk factor for AD based on the studied Tunisian case–control population, made up of a representative sample of 144 patients versus 90 controls, which leads us to conclude that *APOE* ε4 negative AD patients are associated with the nonmemory profile. This conclusion corroborates numerous observations concluding that *APOE* ε4 negative patients are associated with a nonamnestic profile.

**Acknowledgement**

This study was sponsored by The Tunisian Ministry of Public Health, Tunis El Manar university, Faculty of Medicine, Biochemistry and Molecular Biology Laboratory, Children's Hospital. Tunis.

**Authors' contributions**

SF: study concept and design, acquisition of data, analysis and interpretation; AAR: genetic analysis; SB: study supervision; TM: study supervision.

## References

American Psychiatric, Association 2013 *Diagnostic and statistical manual of mental disorders (DSM-5®)*. American Psychiatric Pub.

Ardekani B. A., Izadi N. O., Hadid S. A., Meftah A. M. and Bachman A. H. 2020 Effects of sex, age, and apolipoprotein E genotype on hippocampal parenchymal fraction in cognitively normal older adults. *Psychiatry Res. Neuroimaging* **301**, 111107.

Attia Romdhane N., Ben Hamouda I., Ben Youssef K., Mhenni C., Ouenniche S. and Mrabet A. 2008 Reliability and validity of instrumental activities in daily living scale in Tunisia. *Tunis Med.* **86**, 764–767.

Balasa M., Gelpi E., Antonell A., Rey M. J., Sánchez-Valle R., Molinuevo J. L. *et al*. 2011 Clinical features and APOE genotype of pathologically proven early-onset Alzheimer disease. *Neurology* **76**, 1720–1725.

Bellaj T., Jemaa B. S., Romdhane A. N., Dhiffallah M., Ali B. N., Bouaziz A. M. *et al*. 2008 Version arabe du mini mental state examination (A-MMSE): fidélité, validité et données normatives. *Tunis Med.* **86**, 768–776.

Carrasquillo M., Qu Khan M., Murray M. E., Krishnan S., Aakre J., Pankratz V. S. *et al*. 2014 Late-onset Alzheimer disease genetic variants in posterior cortical atrophy and posterior AD. *Neurology* **82**, 1455–1462.

Christensen D. Z., Schneider-Axmann T., Lucassen P. J., Bayer T. A. and Wirths O. 2010 Accumulation of intraneuronal Abeta correlates with ApoE4 genotype. *Acta Neuropathol.* **119**, 555–566.

Dubois B., Villain N., Frisoni G. B., Rabinovici G. D., Sabbagh M., Cappa S. *et al*. 2021 Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. *Lancet Neurol.* **20**, 484–496.

Fan J., Tao W., Li X., Li H., Zhang J., Wei D. *et al*. 2019 The contribution of genetic factors to cognitive impairment and dementia: apolipoprotein E gene, gene interactions, and polygenic risk. *Int. J. Mol. Sci.* **20**.

Foo H., Thalamuthu A., Jiang J., Koch F., Mather K. A., Wen W. *et al*. 2021 Associations between Alzheimer's disease polygenic

risk scores and hippocampal subfield volumes in 17,161 UK Biobank participants. *Neurobiol. Aging* **98**, 108–115.

Gatz M., Reynolds C. A., Fratiglioni L., Johansson B., Mortimer J. A., Berg S. *et al*. 2006 Role of genes and environments for explaining Alzheimer disease. *Arch Gen. Psychiatry* **63**, 168–174.

Geroldi C., Pihlajamäki M., Laakso M. P., DeCarli C., Beltramello A., Bianchetti A. *et al*. 1999 APOE-epsilon4 is associated with less frontal and more medial temporal lobe atrophy in AD. *Neurology* **53**, 1825–1832.

Goate A., Chartier-Harlin M. C., Mullan M., Brown J., Crawford F., Fidani L. *et al*. 1991 Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704–706.

Hixson J. E. and Vernier D. T. 1990 Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J. Lipid Res.* **31**, 545–548.

Josephs K. A., Duffy J. R., Strand E. A., Machulda M. M., Senjem M. L., Lowe V. J. *et al*. 2014 APOE ε4 influences β-amyloid deposition in primary progressive aphasia and speech apraxia. *Alzheimers Dement* **10**, 630–636.

Kleiman T., Zdanys K., Black B., Rightmer T., Grey M., Garman K. *et al*. 2006 Apolipoprotein E epsilon4 allele is unrelated to cognitive or functional decline in Alzheimer's disease: retrospective and prospective analysis. *Dement. Geriatr. Cogn. Disord.* **22**, 73–82.

Kok E., Haikonen S., Luoto T., Huhtala H., Goebeler S., Haapasalo H. *et al*. 2009 Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. *Ann. Neurol.* **65**, 650–657.

Lehmann M., Ghosh P. M., Madison C., Laforce R., Corbetta-Rastelli C. Jr., Weiner M. W. *et al*. 2013 Diverging patterns of amyloid deposition and hypometabolism in clinical variants of probable Alzheimer's disease. *Brain* **136**, 844–858.

Levy-Lahad E., Wasco W., Poorkaj P., Romano D. M., Oshima J., Pettingell W. H. *et al*. 1995 Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973–977.

Martínez M. F., Martín X. E., Alcelay L. G., Flores J. C., Valiente J. M., Juanbeltz B. I. *et al*. 2009 The COMT Val158 Met polymorphism as an associated risk factor for Alzheimer disease and mild cognitive impairment in APOE 4 carriers. *BMC Neurosci.* **10**, 125.

Mesulam M. M., Johnson N., Grujic Z. and Weintraub S. 1997 Apolipoprotein E genotypes in primary progressive aphasia. *Neurology* **49**, 51–55.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215–1215.

Mishra S., T. Blazey M., Holtzman D. M., Cruchaga C., Su Y., Morris J. C. *et al*. 2018 "Longitudinal brain imaging in preclinical Alzheimer disease: impact of APOE ε4 genotype. *Brain* **141**, 1828–1839.

Novellino F., López M. E., Vaccaro M. G., Miguel Y., Delgado M. L. and Maestu F. 2019 Association between hippocampus, thalamus, and caudate in mild cognitive impairment APOEε4 carriers: a structural covariance MRI study. *Front. Neurol.* **10**, 1303.

Ossenkoppele R., van der Flier W. M., Zwan M. D., Adriaanse S. F., Boellaard R., Windhorst A. D. *et al*. 2013 Differential effect of APOE genotype on amyloid load and glucose metabolism in AD dementia. *Neurology* **80**, 359–365.

Phillips J. S., Da Re F., Irwin D. J., McMillan C. T., Vaishnavi S. N., Xie E. S. X. *et al*. 2019 Longitudinal progression of grey matter atrophy in non-amnestic Alzheimer's disease. *Brain* **142**, 1701–1722.

Pievani M., Rasser P. E., Galluzzi S., Benussi L., Ghidoni R., Sabattoli F. *et al*. 2009 Mapping the effect of APOE epsilon4 on gray matter loss in Alzheimer's disease in vivo. *Neuroimage* **45**, 1090–1098.

Rannikmäe K., Kalaria R. N., Greenberg S. M., Chui H. C., Schmitt F. A., Samarasekera N. *et al*. 2014 APOE associations with severe CAA-associated vasculopathic changes: collaborative meta-analysis. *J. Neurol. Neurosurg. Psychiatry* **85**, 300–305.

Rassas A. A., Mrabet Khiari H., Hadj Fredj S., Sahnoun S., Batti H., Zakraoui N. O. *et al*. 2012 High APOE epsilon 4 allele frequencies associated with Alzheimer disease in a Tunisian population. *Neurol Sci* **33**, 33–37.

Rogalski E. J., Rademaker A., Harrison T. M., Johnson N., Bigio E., Mishra M. *et al*. 2011 ApoE E4 is a susceptibility factor in amnestic but not aphasic dementias. *Alzheimer Dis. Assoc. Disord.* **25**, 159–163.

Saeed U., Mirza S. S., MacIntosh B. J., Herrmann N., Keith J., Ramirez J. *et al*. 2018 APOE-ε4 associates with hippocampal volume, learning, and memory across the spectrum of Alzheimer's disease and dementia with Lewy bodies. *Alzheimers Dement.* **14**, 1137–1147.

Schott J. M., Ridha B. H., Crutch S. J., Healy D. G., Uphill J. B., Warrington E. K. *et al*. 2006 Apolipoprotein e genotype modifies the phenotype of Alzheimer disease. *Arch Neurol*. 155-156.

Sherrington R., Rogaev E. I., Liang Y., Rogaeva E. A., Levesque G., Ikeda M. *et al*. 1995 Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754–760.

Shinohara M., Murray M. E., Frank R. D., DeTure M., Yamazaki Y., Tachibana M. *et al*. 2016 Impact of sex and APOE4 on cerebral amyloid angiopathy in Alzheimer's disease. *Acta Neuropathol.* **132**, 225–234.

Sluimer J. D., Vrenken H., Blankenstein M. A., Fox N. C., Scheltens P., Barkhof F. *et al*. 2008 Whole-brain atrophy rate in Alzheimer disease: identifying fast progressors. *Neurology* **70**, 1836–1841.

Smits L. L., Pijnenburg Y. A., van der Vlies A. E., Koedam E. L., Bouwman F. H., Reuling I. E. *et al*. 2015 Early onset APOE E4-negative Alzheimer's disease patients show faster cognitive decline on non-memory domains. *Eur. Neuropsychopharmacol.* **25**, 1010–1017.

Snowden J. S., Stopford C. L., Julien C. L., Thompson J. C., Davidson Y., Gibbons L. *et al*. 2007 Cognitive phenotypes in Alzheimer's disease and genetic risk. *Cortex* **43**, 835–845.

Tang-Wai D. F., Graff-Radford N. R., Boeve B. F., Dickson D. W., Parisi J. E., Crook R. *et al*. 2004 Clinical, genetic, and neuropathologic characteristics of posterior cortical atrophy. *Neurology* **63**, 1168–1174.

Tiraboschi P., Hansen L. A., Masliah E., Alford M., Thal L. J. and Corey-Bloom J. 2004 Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. *Neurology* **62**, 1977–1983.

Tschanz J. T., Corcoran C. D., Schwartz S., Treiber K., Green R. C., Norton M. C. *et al*. 2011 Progression of cognitive, functional, and neuropsychiatric symptom domains in a population cohort with Alzheimer dementia: the Cache County Dementia Progression study. *Am. J. Geriatr. Psychiatry* **19**, 532–542.

van der Flier W. M., Pijnenburg Y. A., Fox N. C. and Scheltens P. 2011 Early-onset versus late-onset Alzheimer's disease: the case of the missing APOE ε4 allele. *Lancet Neurol.* **10**, 280–288.

van der Flier W. M., Schoonenboom S. N., Pijnenburg Y. A., Fox N. C. and Scheltens P. 2006 The effect of APOE genotype on clinical phenotype in Alzheimer disease. *Neurology* **67**, 526–527.

Warren J. D., Fletcher P. D. and Golden H. L. 2012 The paradox of syndromic diversity in Alzheimer disease. *Nat. Rev. Neurol.* **8**, 451–464.

Whitwell J. L., Dickson D. W., Murray M. E., Weigand S. D., Tosakulwong N., Senjem M. L. *et al*. 2012 Neuroimaging correlates of pathologically defined subtypes of Alzheimer's disease: a case-control study. *Lancet Neurol* **11**, 868–877.

Whitwell J. L., Tosakulwong N., Weigand S. D., Graff-Radford J., Ertekin-Taner N., Machulda M. M. *et al*. 2021 Relationship of APOE, age at onset, amyloid and clinical phenotype in Alzheimer disease. *Neurobiol. Aging* **108**, 90–98.

Yamazaki Yu., Na Zhao, Caulfield T. R., Liu C. and Bu D. 2019 Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat. Rev. Neurol.* **15**, 501–518.

Corresponding editor: B. K. THELMA