Research Article

Association of Apolipoprotein E, Methylenetetrahydrofolate Genotypes, Lipids levels and Alzheimer Disease in an Algerian Population

A. Ouldjaoui[1*], N. Abadi[1,2], K. Sifi[1,2], Y. Sifi[1,3], A. Hamri[1,3], C. Benlatreche[1,2]
[1]Biology laboratory and molecular Genetics, Faculty of Medicine, Constantine, Algeria.
[2]Biochemistry Service, Ibn Badis Hospital, Faculty of Medicine, Constantine, Algeria.
[3]Neurology Service, Ibn Badis Hospital, Faculty of Medicine, Constantine, Algeria.
***Corresponding author's E-mail:** oulahmed@yahoo.fr

Received: 15-11-2015; **Revised:** 22-07-2016; **Accepted:** 14-08-2016.

ABSTRACT

Alzheimer's disease is the most common form of neurodegenerative dementia and affects up to 15 million people worldwide. The aim of the study was to investigate the association between apolipoprotein E, methylenetetrahydrofolate genotypes and lipids as risk factors for Alzheimer disease in a population-based Cross-sectional study of ageing living in Constantine, Algeria. Of an original sample of 187 referrals to a neurology clinic, 63 were given a diagnosis of AD (mean age 73, 47 ± 7, 61 years, mean MMSE 20.0 + 5.7) according to (DSM IV - NINCDS – ADRDA) criteria; and 124 cognitively normal subjects (mean age 67,17 ± 10,84 years). The ApoE allele frequencies of AD cases and controls were 5.5% vs. 7.2% for ε2, 63.5% vs. 83.9% for ε3 and 31% vs. 8.9% for ε4. The AD patients compared with controls subjects had significantly higher mean of total Cholesterol (TC) (191 ± 37 mg/dl vs. 175 ± 37mg/dl, p<0.05), LDL-C (122 ± 29 mg/dl vs. 110 ± 32 mg/dl, p<0.05) levels in men and lower HDL cholesterol with mean values of (37 ± 08 mg/dl vs. 44 ± 07 mg/dl, p<0.05) in men and (42 ± 08 mg/dl vs. 47 ± 08 mg/dl, p<0.05) in women respectively, whereas the triglycerides mean values were no significant in our study population. The carriers of allele ε4 and ε3/ε4 subjects compared with ε3/ε3 are associated with an increased incidence of AD with odds ratio of 5.01[95%CI, 2.36 to 10.71] p<0.001 and 3.86 [95%CI, 1.72 to 8.72] p<0.001, respectively. These results indicated that AD patients with APOE-ε4 allele have a distinct plasma lipid profile and carrier of this allele with high (TC), LDL-C and low levels of HDL-C may be more susceptible to AD. No significant association of *MTHFR* C677T allele and genotype with AD was observed in total samples.

Keywords: Apolipoprotein E, MTHFR, lipids, Alzheimer's disease (AD), Ageing, Genetic polymorphism.

INTRODUCTION

Affecting up to 15 million individuals worldwide, Alzheimer's disease (AD) is the most common form of dementia. Prevalence doubles every 5 years in people over 60 years of age, increasing from 1% among people age 60–64 years to 40% in those age 85 years and older[1]; within ageing populations, prevalence may triple by 2050[2].

The etiopathologenesis of AD is still unclear[3]. Many investigators have shown that apolipoprotein E (APOE)-ε4 allele is a risk factor for AD[4–6]. In addition, increasing evidence suggests that cholesterol plays a key role in the pathogenesis of Alzheimer's disease (AD), the most common cause of senile dementia[7,8]. APOE on chromosome 19 encodes a 299 amino-acid protein with three common isoforms. APOE ε4 differs respectively from APOE ε3 and APOE ε2 by arginine residues instead of cysteine at 112 and 158.

The amino-acid substitutions have a critical role in determining the three-dimensional structure of APOE leading to changes with its protein binding properties. In general, the ε2 isoform is associated with lower plasma cholesterol, while the ε4 allele is associated with higher plasma concentrations of total and LDL cholesterol, as well as a higher risk of atherosclerosis[9].

Inheritance of the ε4 allele is a major risk factor for both sporadic and late-onset AD[10–12]. APOE ε4 is the only genetic marker that has been universally and consistently reported to confer an increased AD risk[13].

Although LDL and HDL levels are well-established risk factors for cardiovascular disease, the relationship of plasma lipid levels to AD risk is far from clear[14].

Several groups have reported that increased midlife total cholesterol (TC) levels are associated with a 2–3-fold increase in AD risk later in life[15,16], and that AD risk is further increased by elevated systolic blood pressure in hypercholesterolemia subjects[17].

Elevated TC and triglyceride (TG) levels have also been reported in subjects with probable/possible AD[18,19]. On the other hand, it was noted that a decrease in serum HDL cholesterol level was correlated with the severity of AD[20]. However, other studies have found no significant associations between plasma TC or HDL levels in AD subjects compared to controls[21,22]. We examined how the APOE-ε4 dose modifies plasma TC, HDL, LDL cholesterol and triglycerides levels in patients with AD and in non-demented elderly subjects.

This study is the first that analyses the distribution of the C677T MTHFR polymorphism in a large group of Algerian patients with AD. Numerous studies[23] reported that patients with cerebrovascular disease have increased levels of homocysteine, so it is hypothesized that the latter may play a role in neurodegenerative disorders. A recent epidemiological study[24] on Japanese population

suggests that APOE ε4 allele and the C677T MTHFR mutation are associated with clinical phenotype and clinical onset of senile dementia.

However, a significant ethnic variation in the C677T MTHFR distribution was demonstrated in several populations, ranging from less than 1% homozygotes to 21% for the T allele (TT genotype)[25].

In light of these findings and on the hypothesis of a population-based susceptibility, we analyzed the distribution of two common polymorphisms, MTHFR C677T and APOE in Algerian patients with sporadic AD.

MATERIALS AND METHODS

Blood samples were drawn after an overnight fasting into EDTA-containing tubes for analysis of ApoE, MTHFR genotypes and lipid parameters. The dosage of cholesterol and triglycerides have been done by an autoanalyser type RA 1000 (Bayer) according to an enzymatic method with commercially available kit (Boehringer Mannheim). HDL-Cholesterol was measured after precipitation of apolipoprotein B containing lipoproteins by the phosphotungstate associated with magnesium chloride[26,27] and the calculation of LDL-Cholesterol was performed by using Friedwald formula[28,29].

Cholesterol LDL = Cholesterol total – Cholesterol HDL – Triglycerides/5.

ApoE and MTHFR Genotyping

DNA was extracted using proteinase K (Sigma) digestion and purified by NaCl. DNA fragment located in the exon 4 encompassing both polymorphisms of the ApoE gene was amplified by polymerase chain reaction (PCR) in a DNA thermal cycler (Genius) using the forward:

(5'- AAC AAC TGC CCC CGG TGG CG -3') and the reverse: (5'- ATG GCG CTG AGG CCG CGC TG-3') primers as previously described[30]. PCR amplification was carried out in a 75µl reaction volume containing 1µl of DNA (250 ng/µl), 200µM of mix dNTP, 3mmol/l $MgCl_2$, 7.5µl DMSO (10%), 7.5µl 10x PCR buffer, 0.6µmol/l of each primer (Eurobio) and 0.5µl (5U/µl) of Taq polymerase (Eurobio). PCR cycling conditions began by one cycle of 5 min for 95°C, followed by 5 min for 65°C. Then a series of 30 cycles at 72°C for 50s, 94°C for 50s and 65°C for 50s, followed by a final extension phase of 10 min at 72°C. 15µl of PCR products were controlled by electrophoresis on 1.5% agarose gel to confirm the presence of the amplified ApoE DNA fragment which is a 292 bp. The PCR products were digested with restriction enzyme HhaI (Sigma), using 1µl of enzyme (10U/µl) for 25µl of the amplified product, and were incubated for 4 hrs at 37°C.

Fragments of enzymatic restriction were separated by electrophoresis in polyacrylamide gel (10%) for 2-3 hrs at 250V.

After electrophoresis, the gel was treated with ethidium bromide for 15 min and visualized by UV illumination. Fragments sizes were estimated by a known size bp marker pBR322 HaeIII digest (Sigma).

The MTHFR gene was amplified by a polymerase chain reaction (PCR)[31] using the following primers: 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' and 5'-AGG ACG GTG CGG TGA GAG TG-3'.

30µl of PCR product were digested with HinfI according to the manufacturer's protocol (Amersham, Arlington Heights, IL; USA) in a final volume of 40µl. After digestion, samples were loaded on a 3% agarose gel, and DNA fragments were visualized with ethidium bromide.

Statistical Analysis

Data of age and lipid levels are reported as mean ± S.D. The software Epi Info and SPSS for Windows version 10.0 statistical packages were used.

The Pearson x2-test was used to compare categorical variables. We tested the age-distribution between the groups by using one-way analysis of variance method. Sex between groups was tested by using Pearson x2-test. Means were compared by ANOVA.

The correlation between quantitative lipid levels and dementia was tested by one-way analysis of variance method, ANOVA.

RESULTS

The ApoE and MTHFRC677T genotypes distribution and allele's frequencies in AD patients as well as in controls subjects are displayed in (**Table 1)**. The three common alleles of apo E were detected, whereas Apo ε2/ε2 genotype was not found among study subjects. The frequencies of apo E genotypes (ε3/ε3, ε3/ε4, ε2/ε3, ε2/ε4 and ε4/ε4) and alleles (ε2/ε3/ε4) were 69.4/15.3/13.7/0.8/0.8 and 7.2/83.9/8.9 in the control group, and 42.9/36.5/4.8/6.3/9.5 and 5.5/63.5/31 in the patient group, respectively. ApoE genotypes were in Hardy-Weinberg Equilibrium HWE for AD patients and controls.

There was a statistically significant difference between AD patients and controls in the distribution of Apo E genotypes and alleles.

A higher frequency of the Apo ε4 allele (31% vs. 8.9%) and slightly lower frequency of the Apo ε3 allele (63.5% vs. 83.9%) were observed in the AD patients compared with the control subjects, therefore there was no difference in the distribution of the ε2 allele between AD patients and control (5.5% vs. 7.2%).

AD patients have significantly higher frequencies of genotypes ε3/ε4 (36.5% vs. 15.3%), ε4/ε4 (9.5% vs. 0.8%) and ε2/ε4 (6.3% vs. 0.8%) and lower frequencies of genotypes ε3/ε3 (42.9% vs. 69.4%) and ε2/ε3 (4.8% vs. 13.7%).

The odds ratio (OR) indicate that of ε4 (ε2/ε4, ε3/ε4, ε4/ε4) subjects vs. ε3/ε3, and ε3/ε4 vs.ε3/ε3 present a significant association with the incidence of AD with odds

ratio 5.01 [95%CI, 2.36 to 10.71], p<0.001 and 3.86 [95%CI, 1.72 to 8.72], p<0.001, respectively, whereas the odds ratio for ε2 vs. ε3/ε3 and ε2/ε3 vs. ε3/ε3 were no significant (**Table 2**).

The distribution of a number of ε4 copies in study population shows lower frequencies in AD patients lacking the apoE-ε4 allele compared to control subjects (22.5% vs. 77.5%), and higher frequencies in the AD subjects with one, one or two and two copies of the apoE-ε4 allele (57.4% vs. 42.6%, 0R = 4.64, p<0.001), (61.1% vs. 38.9%, OR = 5.40, p<0.001) and (85.7% vs. 14.3%, OR = 20.6, p<0.001) respectively (**Table 3**).

Lipid levels in AD and in controls are presented in **(Table 4)**. Compared with controls, the AD subjects had significantly higher serum level of TC (191 ± 37 mg/dl versus 175 ± 37 mg/dl, *p*< 0.05) and LDL-C (122 ± 29 mg/dl versus 110 ± 32 mg/dl, *p*< 0.05) in men, but significantly lower levels of HDL-C (37 ± 08 mg/dl versus 44 ± 07 mg/dl, *p* < 0.05), and (42 ± 08 mg/dl versus 47 ± 08 mg/dl, p<0.05) in men and women respectively, whereas the triglycerides mean values were no significant in our study population.

The frequencies of MTHFR genotypes (CC/CT/TT) and alleles (C/T) were 42.7/46.8/10.5 and 66/34 in the control group, and 30.2/49.2/20.6 and 54.8/45.2 in the AD cases, respectively. MTHFRC677T genotypes were also in (HWE) for AD patients and controls.

The distribution of *MTHFR*C677T alleles and genotypes in AD with and without ε4 allele is displayed in (**Table 5**). No significant difference in *MTHFR* C677T allele and genotype frequencies between the AD cases carriers and non carriers of APOE ε4 allele were detected in the total samples.

The T allele frequency and TT genotype distributions in the AD cases were no significant (T vs. C: Chi^2 = 0.01 *p* = 0.97; TT vs. CC plus CT: chi^2 = 0.65, *p* = 0.41) in the *APOE* ε4 (+) subgroups.

Table 1: Repartition of the genotypes and allele frequencies of ApoE and MTHFRC677T in control subjects and subjects with AD

	Controls		AD	
	n	***%***	**n**	***%***
ε3/ε3	86	*69.4*	27	*42.9*
ε3/ε4	19	*15.3*	23	*36,5*
ε2/ε3	17	*13.7*	3	*4.8*
ε2/ε4	1	*0.8*	4	*6.3*
ε4/ε4	1	*0.8*	6	*9.5*
ε2	18	7.2	7	5.5
ε3	208	83.9	80	63.5
ε4	22	8.9	39	31
CC	*53*	*42.7*	*19*	*30.2*
CT	58	*46.8*	31	*49.2*
TT	13	*10.5*	13	*20.6*
C	164	*66*	69	*54.8*
T	84	*34*	57	*45.2*

Table 2: Calculating the odds ratio of AD patients having a ε4, ε2, ε3/ε4 and ε2/ε3 allele compared to controls subjects with ε3/ε3 genotype.

	Odds ratio	p
ε4 vs ε3/ε3	**5.01** (2.36<OR<10.71*)	**<0.001**
ε2 vs ε3/ε3	**1.24** (0.42<OR<3.59*)	**ns**
ε3/ε4 vs ε3/ε3	**3.86** (1.72<OR<8.72*)	**<0.001**
ε2/ε3 vs ε3/ε3	**0.56** (1.12<OR<2.26*)	**ns**

Confidence interval (95% CI)

Table 3: Distribution according to % of AD and a number of ε4 copies

number of ε4 copies	AD	Controls	% AD	% Controls	OR	p
0	30	103	**22.5**	**77.5**		
1	27	20	**57.4**	**42.6**	4.64	<0.001
1 or 2	33	21	**61.1**	**38.9**	5.40	<0.001
2	6	1	**85.7**	**14.3**	20.6	<0.001

Table 4: Comparison between Control Subjects and AD with Respect to Serum Lipids

	Male		Female	
	Controls	AD	Controls	AD
TC (mg/dl)	175 ± 37	191 ± 37*	182 ± 39	187 ± 39
TG (mg/dl)	117 ± 57	129 ± 72	119 ± 67	122 ± 37
C-HDL	44 ± 07	37 ± 08*	47 ± 08	42 ± 08*
C-LDL	110 ± 32	122 ± 29*	115 ± 34	114 ± 34

*P<0.05

Table 5: Distribution of the MTHFR C677T alleles and genotypes in the AD cases

Genotypes and Alleles Frequency	AD APOE4 (+)	AD APOE4 (–)	Chi^2	p
CC	08 **(24.2%)**	10 **(33.3%)**		
CT	19 **(57.6%)**	12 **(40%)**		
TT	06 **(18.2%)**	08 **(26.7%)**	0.65	0.41
C	35 **(53%)**	32 **(53.3%)**		
T	31 **(47%)**	28 **(46.7%)**	0.01	0.97

APOE ε4 (+): subjects who contain one or two ε4 alleles; APOE ε4 (−): subjects who do not contain ε4 allele.

DISCUSSION

There is nonetheless a real consensus about the importance of the genetic component on the risk of AD. In most cases, AD is a complex multifactorial disease resulting from the interaction of several factors - principally genetic but also environmental.

The 1993 discovery that the apolipoprotein E4 (ApoE4) allele is genetically associated with increased risk in both sporadic and familial late-onset AD strongly supports the validity of this genetic approach. That study showed a frequency of the ε4 allele as high as 40% in a population with late-onset familial forms of AD[32].

The current study specifically aimed to examine whether APOE, MTHFRC677T polymorphisms in association with serum lipids level are risk factors for AD in a population from Constantine, Algeria. The APOE gene has three major alleles in the general population, ε2, ε3 and ε4.

The ε3 allele is characterized by arginine at codon 112 and cysteine at codon 158, while the ε4 allele differs only by arginine at codon 158 and the ε2 allele by cysteine at codon 112. The corresponding isoforms are ApoE2, ApoE3 and ApoE4. Although the ε4 allele appears to be the ancestral allele, ε3 is most frequent in white populations (around 80%). The ε2 and ε4 alleles are less frequent, appearing in 8% and 12% of these populations.

Studies conducted in different parts of the globe reveal that the gene frequencies at apo E locus are highly heterogeneous between the populations. The ε3 is the most common form of the gene in most of the populations followed by ε4 and ε2 alleles[33]. In the present study, apo E allele frequencies in the control group and AD cases are similar to those found for Southern Europeans: French[34], Spanish[35], and Asians[36]. Significant differences were found between our results and those of Chinese population[37] where the ε4 allele was the lowest.

In this study, there was a significant interaction between serum lipids level, APOE-ε4, and the risk of AD, which is very similar to the report on African Americans[38]. Increasing levels of cholesterol were associated with an increased risk of AD, but only for individuals without the ε4 allele. A similar interaction with ε4 and AD risk was seen for LDL where increasing levels of LDL were also associated with an increased risk of AD. There was no significant interaction between triglycerides, *APOE*, and AD risk in our study[39]. Consequently increased levels of triglycerides were associated with an increase in the risk of AD[40]. The *APOE* and lipid interaction is being explored

as an explanation to understand the risk of AD. Some studies have reported a significant interaction between *APOE* and cholesterol in determining the risk of AD[41,42]. One of these studies suggested that cholesterol, in fact, mediates some of the effects of *APOE*-ε4 on AD[42]. However, the reports on cholesterol levels and AD risk have not always been consistent. Some studies have failed to find a relationship between cholesterol and AD risk[43] or an interaction between *APOE*, cholesterol, and AD[44].

In general, the ε2 isoform is associated with lower plasma cholesterol, while the ε4 allele is associated with higher plasma concentrations of total and LDL cholesterol, as well as a higher risk of atherosclerosis[9]. Inheritance of the ε4 allele is a major risk factor for both sporadic and late-onset AD[45-47]. *APOE* ε4 is the only genetic marker that has been universally and consistently reported to confer an increased AD risk[47]. In AD, the ε4 genotype lowers the age at onset of dementia in a gene-dose-dependent manner by as much as 7–9 years per allele[45]. The risks of AD are three and eight times greater in individuals with one or two copies of the ε4 gene respectively, compared with people homozygous for ε3[46]. In most studies, 40–50% of patients with AD have at least one ε4 allele, compared with 10–15% of healthy controls[12,45]. Individuals who are homozygous for the ε4 allele and live to age 80 years will almost invariably develop AD, but about 10% of heterozygous ε4 carriers will remain free of AD well into their 80s[12,47].

Studies of elevated LDL cholesterol level in carriers with the *APOE*-ε4 allele[48] suggested that the *APOE*-ε4 allele in itself decreases plasma HDL cholesterol level as well as increases plasma LDL cholesterol level, independently of nutrition. Elevated serum LDL cholesterol level, related to the *APOE*-ε4 allele, is correlated with brain Aβ 1– 42 levels[49]. On the other hand, a correlation between plasma HDL cholesterol and dementia has been reported[20]. The soluble form of Aβ is associated with HDL in not only human plasma but also in cerebrospinal fluid[50], and HDL cholesterol increased degradation of Aβ in a dose-dependent manner *in vitro*[51]. The major apolipoproteins in HDL cholesterol, apolipoprotein A1 and A2, are not synthesized in the brain, and are transported from blood to the cerebrospinal fluid[52].

Therefore, plasma HDL cholesterol level, modified by the *APOE*-ε4 dose, is thought to reflect HDL cholesterol level in cerebrospinal fluid, and is related to the potential to degrade Aβ in the brain. Therefore, plasma lipid likely modifies the development of AD.

As shown in Table 1, among the 63 AD patients, 20.6% were homozygous (TT) for MTHFR and 49.2% were heterozygous (CT), we did not find significant differences (10.5% TT 46.8% CT) in the control group. There were no statistically significant differences in the allele and genotype distributions in AD patients compared with controls. We found the combination of the MTHFR/TT and ApoE ε4/ε4 genotypes in two cases among the 63 AD.

Our data do not provide evidence for an association between the MTHFR C677T mutation and AD. This investigation on 63 patients extends and confirms three earlier reports (45 AD patients in Zuliani and colleagues[53]; 49 AD patients in Chapman[54]; and 140 AD patients in Regland[55]).

One study confirms the association between the C677T MTHFR mutation and Vascular Dementia[56]. Seripa found no difference in MTHFR polymorphism distribution between AD cases and elderly controls in both American cohort and Italian cohort[57].

Religa found that plasma total homocysteine is increased in AD patients and depended on the MTHFR T/T genotype (mutation homozygote) in the presence of low folate levels; however the distribution of MTHFR C677T polymorphism in the Polish population does not differ in AD and controls[58].

Our negative results, in individuals with Probable AD, confirmed the lack of association between AD and C/T polymorphism in the MTHFR gene.

CONCLUSION

This finding has demonstrated that the APOE-ε4 allele is associated with increased risk of AD, in Algerian population. The APOE-ε4 affects the composition of plasma cholesterol.

We have observed that AD patients with APOE-ε4 allele have a distinct plasma lipid profile. Relative to ε3/ε3 individuals, ε2 allele was a protective factor for AD; the presence of allele ε4 demonstrated a dose-dependent effect, increasing exponentially compared with genotype ε2/ε3; there was a discreet tendency of MTHFR allele T presence in AD patients adjusted to ε4 allele effect, but it was not significant; the MTHFR mutation did not demonstrate significant difference in cases and controls. The *MTHFR* 677 T allele and the *APOE* ε4 allele may synergistically act to increase the risk of AD. Further analysis of samples from different ethnicities or communities need to be done to confirm the associations.

Acknowledgement: This study was sponsored by the Laboratory of Biology and molecular Genetics, Medicine Faculty, and the Neurology service Ibn Badis Hospital Constantine, Algeria.

REFERENCES

1. Von Strauss E, Viitanen M, De Ronchi D, Winblad B, Fratiglioni L. Aging and the occurrence of dementia: findings from a population based cohort with a large sample of nonagenarians. Arch Neurol; 56, 1999, 587–92.
2. Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset, Am J Public Health; 88, 1998, 1337-42.
3. Bonarek M, Barberger-Gateau P, Letenneur L, Deschamps V, Iron A, Dubroca B, Dartigues J.F. Relationships between cholesterol, apolipoprotein E polymorphism and dementia: a cross-sectional

analysis from the PAQUID Study, Neuroepidemiology; 19, 2000, 141–148.

4. de-Andrade F.M, Larrandaburu M, Callegari-Jacques S.M, Gastaldo G, Hutz M.H. Association of apolipoprotein E polymorphism with plasma lipids and Alzheimer's disease in a Southern Brazilian population, Braz. J. Med Biol Res; 33, 2000, 529–537.
5. Khachaturian A.S, Corcoran C.D, Mayer L.S, Zandi P.P, Breitner J.C. Cache County Study Investigators, Apolipoprotein E epsilon 4 count affects age at onset of Alzheimer disease, but not lifetime susceptibility: the Cache County Study, Arch Gen Psychiatry; 61(5), 2004, 518–524.
6. Raygani A.V, Zahrai M, Raygani A.V, Doosti M, Javadi E, Rezaei M. Pourmotabbed T. Association between apolipoprotein E polymorphism and Alzheimer disease in Tehran, Iran, Neurosci Lett; 375, 2005, 1–6.
7. Casserly I, Topol E. Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins, Lancet; 363, 2004, 1139–1146.
8. Wolozin B. Cholesterol and the biology of Alzheimer's disease, Neuron; 41, 2004, 7–10.
9. Murakami K, Shimizu M, Yamada N, Ishibashi S, Shimano H, Yazaki Y, Akanum Y. Apolipoprotein E polymorphism is associated with plasma cholesterol response in a 7-day hospitalization study for metabolic and dietary control in NIDDM. Diabetes Care; 16, 1993, 564–69.
10. Luthra K, Tripathi M, Grover R, Dwivedi M, Kumar A, Dey AB. Apolipoprotein E gene polymorphism in Indian patients with Alzheimer's disease and Indian patients with Alzheimer's disease and vascular dementia. Dement Geriatr Cogn Disord; 17, 2004, 132–5.
11. Graff-Radford NR, Green RC, Go RC, Hutton ML, Edeki T, Bachman D, Adamson JL, Griffith P, Willis FB, Williams M, Hipps Y, Haines JL, Cupples LA, Farrer LA. Association between apolipoprotein E genotype and Alzheimer disease in African American subjects. Arch Neurol; 59, 2002, 594–600.
12. Hsiung GY, Sadovnick AD, Feldman H. Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: data from the Canadian Study of Health and Aging. CMAJ; 171, 2004, 863–67.
13. Slooter AJ, Cruts M, Kalmijn S, Hofman A, Breteler MM, Van Broeckhoven C, van Duijn CM. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol; 55, 1998, 964–68.
14. Hoglund K, Thelen K.M, Syversen S, Sjogren M, Von Bergmann K, Wallin A, Vanmechelen E, Vanderstichele H, Lutjohann D, Blennow. The effect of simvastatin treatment on the amyloid precursor protein and brain cholesterol metabolism in patients with Alzheimer's disease. Dement Geriatr Cogn Disord; 19, 2005, 256–265.
15. Kivipelto M, Helkalal E.-L, Laakso M, Hänninen T, Hallikainen M, Alhainen K, Ilvonen S, Mannermaa A, Tuomilehto J, Nissinen A, Solninen H. Apolipoprotein E e4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. Ann Intern Med; 137, 2002, 149–155.
16. Whitmer R.A, Sidney S, Selby J, Claibourne Johnston S, Yaffe K. Midlife cardiovascular risk factors and risk of dementia in late life. Neurology; 64, 2005, 277–281.
17. Kivipelto M, Helkalal E.-L, Laakso M.P, Hänninen T, Hallikainen M, Alheinen K, Soininen H, Tuomilehto J, Nissien A. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. BMJ; 322, 2001, 1447–1451.
18. Sabbagh M, Sahiri H.R, Ceimo J, Cooper K, Gaul W, Connor D, Sparks D.L. Is there a characteristic lipid profile in Alzheimer's disease? J Alzheimer's Dis; 6, 2004, 585–589.
19. Suryadevara V, Storey S.G, Aronow W.S, Ahn C. Association of abnormal serum lipids in elderly persons with atherosclerotic vascular disease and dementia, atherosclerotic vascular disease without dementia, dementia without atherosclerotic vascular disease, and no dementia or atherosclerotic vascular disease. J. Gerontol; A Biol Sci Med Sci; 58, 2003, M859–M861.
20. Merched A, Xia Y, Visvikis S, Serot J.M, Siest G. Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease. Neurobiol Aging; 21, 2000, 27–30.
21. Reitz C, Tang M.X, Luchsinger J, Mayeux R. Relation of plasma lipids to Alzheimer disease and vascular dementia. Arch Neurol; 61, 2004, 705–714.
22. Zaldy S.T, Seshardri S, Beiser A, Wilson P.W.F, Kiel D.P, Tocco M, D'Agostino R.B, Wolf P.A. Plasma total cholesterol level as a risk factor for Alzheimer disease. Arch Intern Med; 163, 2003, 1053–1057.
23. Stampfer M.J, Malinow M.R, Willett W.C, Newcomer L.M, Upson B, Ullmann D, Tishler P.V and Hennekens C.H. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians, J Am Med Assoc; 268, 1992, 877–881.
24. Nishiyama M, Kato Y, Hashimoto M, Yukawa S and Omori K. Apolipoprotein E, methylenetetrahydrofolate reductase mutation and risk of senile dementia-an epidemiological study using the polymerase chain reaction (PCR) method, J Epidemiol; 10, 2000, 163–172.
25. Stevenson R.E, Schwartz C.E, Du Y.Z and Adams Jr M.J. Differences in methylenetetrahydrofolate reductase genotype frequencies, between Whites and Blacks, Am J Hum Genet; 60, 1997, 229–230.
26. Arcol/SFBC. Commissions Standardization lipids-lipoproteins: Option/Bio.Suppl; 49.1, 1996, 172.
27. Masson D, Mainard F, Auget J.L and Chivot L. Effect of precipitation and centrifugation conditions on high-density lipoprotein cholesterol measured with phosphotungstate-magnesium reagent, Clin Chem; 38/1, 1992, 148.
28. Lippi U, Graziani M.S, Manzat F and Schinella M. The Friedewald formula: statistical acrobatics? Clin Chem; 32, 1986, 909-911.
29. Friedwald W.T, Levy R.I and Fredrickson D.S. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin Chem; 18, 1972, 499-502.
30. Richard P, Thomas G, de Zulueta M P, De Gennes J L, Thomas M, Cassaigne A, Béréziat G, Iron A. Common and rare genotypes of human apolipoprotein E determined by specific restriction profiles of polymerase chain reaction-amplified DNA. Clin Chem; 40, 1994, 24-29.
31. Abbate R, Sardi I, Pepe G, Marcucci R, Brunelli T, Prisco D, Fatini C, Capanni M, Simonetti I and Gensini G.F. The high prevalence of thermolabile 5-10 methylenetetrahydrofolate reductase (MTHFR) in Italians is not associated to an increased risk for coronary artery disease (CAD), Thromb Haemost; 79, 1998, 727–730.
32. Strittmatter W.J, Saunders A.M, Schmechel D, Pericak-Vance M, Enghild J, Salvesen G.S, Roses A.D. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA; 90, 1993, 1977–1981.
33. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol; 155, 2002, 487–495.

34. Bailleul S, Couderc R, Landais V, Lefevre G, Raichvarg D, Étienne J. Direct phenotyping of human apolipoprotein E in plasma: application to population frequency distribution in Paris (France). Human Heredit; 43, 1993, 159-65.

35. Adroer R, Santacruz P, Blesa R, Lopez-Pousa S, Ascaso C, Oliva R. Apolipoprotein E4 allele frequency in Spanish Alzheimer and control cases. Neurosci Lett; 189, 1995, 182-6.

36. Tsukamoto K, Watanabe T, Matsushima T, Kinoshita M, Kato H, Hashimoto Y, Kurokawa K, Teramoto T. Determination by PCR-RFLP of apo E genotype in a Japanese population. J Lab Clin Med; 121, 1993, 598-602.

37. Shen X, Xia Y, Sasse C, Visvikis S, Siest G. Association of apolipoprotein E polymorphism and concentration with serum lipids and apolipoprotein level in the Chinese from Shanghai. Clin Chem Lab Med; 36, 1998, 615-9.

38. Evans RM, Emsley CL, Gao S, Sahota A, Hall KS, Farlow MR, Hendrie H. Serum cholesterol, APOE genotype and the risk of Alzheimer disease in a population-based study of African Americans. Neurology; 54, 2000, 240–242.

39. Francesco Panzaa, Alessia D'Introno, Anna Maria Colacicco, Cristiano Capursob, Gianfranco Pichichero. Lipid metabolism in cognitive decline and dementia, Brain research reviews; 51, 2006, 275-292.

40. Francesco Panzaa. Serum total cholesterol as a biomarker for Alzheimer's disease: Mid-life or late-life determinations? Experimental Gerontology; 41, 2006, 805-806.

41. Dufouil C, Richard F, Fiévet N, Dartigues JF, Ritchie K, Tzourio C, Amouyel P, Alpérovitch A. APOE genotype, cholesterol level, lipid-lowering treatment, and dementia: the Three-City Study. Neurology; 64, 2005, 1531–1538.

42. Notkola IL, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, Tuomilehto J, Nissinen A. Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. Neuroepidemiology; 17, 1998, 14–20.

43. Mielke MM, Zandi PP, Sjögren M, Gustafson D, Östling S, Steen B and. Skoog I. High total cholesterol levels in late life associated with a reduced risk of dementia. Neurology; 64, 2005, 1689–1695.

44. Romas SN, Tang MX, Berglund L, Mayeux R. APOE genotype, plasma lipids, lipoproteins, and AD in community elderly. Neurology; 53, 1999, 517–521.

45. Jarvik GP, Wijsman EM, Kukull WA, Schellenberg GD, Yu C, Larson EB. Interactions of apolipoprotein E genotype, total cholesterol level, age, and sex in prediction of Alzheimer's disease: a case-control study. Neurology; 45, 1995, 1092–96.

46. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science; 261, 1993, 921–23.

47. Rubinsztein DC, Easton DF. Apolipoprotein E genetic variation and Alzheimer's disease. A meta-analysis. Dement Geriatr Cogn Disord; 10, 1999, 199–209.

48. Wehr H, Parnowski T, Puzynski S, Bednarska-Makaruk M, Bisko M, Kotapka-Minc S, Rodo M, Wolkowska M. Apolipoprotein E genotype, and lipid, and lipoprotein levels in dementia. Dement Geriatr Cogn Disord; 11, 2000, 70–3.

49. Kuo YM, Emmerling MR, Bisgaier CL, Essenburg AD, Lampert HC, Drumm D, Roher AE. Elevated low-density lipoprotein in Alzheimer's disease correlates with brain Aβ 1–42 level. Biochem Biophys Res Commun; 252, 1998, 711–5.

50. Koudinov AR, Koudinova NV, Kumar A, Beavis RC, Ghiso J. Biochemical characterization of Alzheimer's soluble amyloid beta protein in human cerebrospinal fluid: association with high density lipoproteins. Biochem Biophys Res Commun; 223, 1996, 592–7.

51. Cole GM, Beech W, Frautschy SA, Sigel J, Glasgow C, Ard MD. Lipoprotein effects on A accumulation and degradation by microglia in vitro. J Neurosci Res; 57, 1999, 504–20.

52. Montine KS, Bassett CN, Ou JJ, Markesbery WR, Swift LL, Montine TJ. Apolipoprotein E allelic influence on human cerebrospinal fluid apolipoproteins. J Lipid Res; 39, 1998, 2443–51.

53. Zuliani G, Ble A, Zanca R, Munari M.R, Zurlo A, Vavalle C, Atti A.R and Fellin R. Genetic polymorphisms in older subjects with vascular or Alzheimer's dementia, Acta Neurol. Scand; 103, 2001, 304–308.

54. Chapman J, Wang N, Treves T.A, Korczyn A.D and Bornstein N.M. ACE, MTHFR, Factor V Leiden, and APOE polymorphism in patients with vascular and Alzheimer's dementia. Stroke; 29, 1998, 1401–1404.

55. Regland B, Blennow K, Germgerd T, Kech-Schmidt A.C and Gottfries C.G. The role of the polymorphic genes Apolipoprotein E and Methylene-tetrahydrofolate reductase I the development of dementia of the Alzheimer type, Dement Geriatr Cogn Disord; 10, 1999, 245–251.

56. Pollak R.D, Friedlander Y, Pollak A, Idelson M, Bejarano- Achache I and Blumenfeld A. Ethnic differences in the frequency of the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene in healthy Israeli populations, Genet Test; 4, 2000, 309–311.

57. Gravina C, Margaglione M, Palermo MT, Wekstein DR, Antuono P, Davis DG, Daniele A, Masullo C, Bizzarro A, Gennarelli M, Fazio VM. Methylenetetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms in two genetically and diagnostically distinct cohort of Alzheimer patients. Neurobiol Aging; 24, 2003, 933-939.

58. Religa D, Styczynska M, Peplonska B, Gabryelewicz T, Pfeffer A, Chodakowska M, Luczywek E, Wasiak B, Stepien K, Golebiowski M, Winblad B, Barcikowska M. Homocysteine, apolipoproteine E and methylenetetrahydrofolate reductase in Alzheimer's disease and mild cognitive impairment. Dement Geriatr Cogn Disord; 16, 2003, 64-70.

Source of Support: Nil, **Conflict of Interest:** None.