Genes Nutr (2014) 9:393
DOI 10.1007/s12263-014-0393-7

RESEARCH PAPER

Genotype patterns at *CLU*, *CR1*, *PICALM* and *APOE*, cognition and Mediterranean diet: the PREDIMED-NAVARRA trial

Elena H. Martínez-Lapiscina · Cecilia Galbete · Dolores Corella · Estefania Toledo · Pilar Buil-Cosiales · Jordi Salas-Salvado · Emilio Ros · Miguel Á. Martinez-Gonzalez

Received: 13 December 2013 / Accepted: 26 February 2014 / Published online: 19 March 2014

Abstract The traditional Mediterranean diet (MedDiet) has shown beneficial effects on cognitive decline. Nevertheless, diet–gene interactions have been poorly evaluated. We aimed to investigate diet–gene interaction in the PREDIMED-NAVARRA randomized trial. A total of 522 participants (67 ± 6 years at baseline) enrolled in the PREDIMED-NAVARRA trial were randomly allocated to one of three diets: two MedDiets (supplemented with either extra-virgin olive oil or nuts) or a low-fat diet. They were evaluated with the Mini-Mental State Examination (MMSE) and the Clock Drawing Test (CDT) after 6.5 years of intervention. Subjects were genotyped for *CR1*-rs3818361, *CLU*-rs11136000, *PICALM*-rs3851179 and *Apolipoprotein E* (*ApoE*) genes. We studied MedDiet–gene interactions for cognition and assessed the effect of the MedDiet on cognition across different genetic profiles. A significant interaction ($p = 0.041$) between *CLU*-rs11136000 and the MedDiet intervention on the MMSE was found with a beneficial effect of MedDiet among carriers of the T minor allele ($B = 0.97$, 95 % CI 0.45–1.49). Similar effect was observed for *CR1*-rs3818361, but no significant interaction was observed ($p = 0.335$). For *PICALM*-rs3851179, the MedDiet intervention showed a beneficial effect in both genotype groups. No apparent interaction was found for the CDT between intervention and gene variants. Similarly, participants randomly allocated to MedDiet groups, with favorable

Trial Registration-ISRCTN: 35739639.

Elena H. Martínez-Lapiscina and Cecilia Galbete have contributed equally to this work.

On behalf of the PREDIMED Group.

PREDIMED Group collaborators names are mentioned in "Appendix" section.

E. H. Martínez-Lapiscina · C. Galbete · E. Toledo · P. Buil-Cosiales · M. Á. Martinez-Gonzalez (✉)
Departamento de Medicina Preventiva y Salud Pública, Facultad de Medicina-Clínica Universidad de Navarra, Universidad de Navarra, C/Irunlarrea n1 1, Pamplona, Navarra 31008, Spain
e-mail: mamartinez@unav.es

E. H. Martínez-Lapiscina
Center for Neuroimmunology, Institute of Biomedical Research August Pi Sunyer (IDIBAPS), Hospital Clinic of Barcelona, Barcelona, Spain

D. Corella
Department of Preventive Medicine, University of Valencia, Valencia, Spain

D. Corella · E. Toledo · P. Buil-Cosiales · J. Salas-Salvado · E. Ros · M. Á. Martinez-Gonzalez
CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Barcelona, Spain

P. Buil-Cosiales
Navarra Public Health Service (Osasunbidea, Servicio Navarro de Salud), Pamplona, Spain

J. Salas-Salvado
Human Nutrition Unit, Faculty of Medicine and Health Sciences, IISPV, Universitat Rovira i Virgili, Reus, Spain

E. Ros
Lipid Clinic, Endocrinology and Nutrition Service, Institut d'Investigacions Biomédiques August Pi i Sunyer (IDIBAPS), Hospital Clinic, Barcelona, Spain

profiles of *CR1*, *CLU* and *PICALM* genes, significantly improved CDT scores compared to controls with the same genetic profile. Cognitive performance was better for non-*ApoE4* and for *ApoE4* carriers of MedDiet groups compared to controls, but for CDT performance, we only found statistical significant differences for non-*ApoE4* carriers. A MedDiet intervention modulates the effect of genetic factors on cognition. The effect of MedDiet might be greater for subjects with a more favorable genetic profile.

Keywords Mediterranean diet · Cognition · *CLU* · *CR1* · *PICALM* · *APOE*

Introduction

More than 35.6 million people were living with dementia worldwide in 2010 (Prince et al. 2013). Due to the increased life-expectancy, worldwide prevalence of dementia is projected to be 115.4 million in 2050 (Prince et al. 2013). Alzheimer's disease (AD), vascular dementia (VD) and a mixed neurodegenerative–vascular dementia are the most common types of dementia (Jellinger and Attems 2010). There is not cure for dementia. Vascular dementia can be delayed or even prevented by treatment or management of the predisposing vascular factors (Chang-Quan et al. 2011; Exalto et al. 2012), but currently, there is no available treatment for delaying the onset or the progression of Alzheimer disease. Moreover, the only dementia with specific therapies is the dementia caused by Alzheimer's Disease, and these treatments offer only symptomatic relief (Birks 2006; McShane et al. 2006). Thus, dementia is a public health priority, and prevention is an essential strategy to deal with it.

Apolipoprotein E (*ApoE*) is the most important susceptibility locus for late-onset AD (Burke and Roses 1991), and it has been also related to a higher risk of VD (Liu et al. 2012; Yin et al. 2012). Most recently, genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) at the *CLU*, *CR1* and *PICALM* loci that increase the risk of late-onset AD (Harold et al. 2009; Lambert et al. 2009). A higher educational level and higher engagement in physical, cognitive and social activities are lifestyle factors that may reduce the risk of dementia or cognitive decline (Hamer and Chida 2009; Kareholt et al. 2011; Morgan et al. 2012; Niti et al. 2008; Sattler et al. 2012). Nutritional epidemiology has examined the potential benefit on cognition of fatty acids (van Gelder et al. 2007), vitamins (Mangialasche et al. 2010; Tangney et al. 2009), fish (van Gelder et al. 2007), fruit and vegetables (Hughes et al. 2010) with favorable but not fully consistent results. Thus, both genetic and lifestyle-related factors are involved in cognitive decline and dementia, but these factors do not contribute independently to neurodegeneration. In fact, the development and also the phenotype of neurodegenerative processes are a result of complex gene–environment interactions. The interaction between *ApoE* genotype and physical and cognitive activities (Niti et al. 2008) and some nutrients (Barberger-Gateau et al. 2007) has been examined with conflicting results.

Over the last few years, interest in overall dietary patterns has risen to a level comparable to the interest in nutrients and foods. The Mediterranean diet (MedDiet) pattern is characterized by olive oil as the main culinary fat, high intake of plant-based, moderate-to-high consumption of fish and seafood, moderate-to-low intake of dairy products, low intake of meat or meat products and regular but moderate intake of red wine during meals (Willett et al. 1995). Compelling evidence worldwide supports a benefit effect of this emerging nutritional pattern on health (Roman et al. 2008). The MedDiet has provided favorable results on cognitive function, cognitive decline and dementia prevention along several observational studies (Scarmeas et al. 2006; Psaltopoulou et al. 2008; Gu et al. 2010; Feart et al. 2009; Scarmeas et al. 2009a, b; Roberts et al. 2010; Tangney et al. 2011; Kesse-Guyot et al. 2013; Samieri et al. 2013; Tsivgoulis et al. 2013). Recently, the PREDIMED-NAVARRA primary prevention trial has confirmed the benefit on cognitive function and mild cognitive impairment development (MCI) of a nutritional intervention with MedDiet supplemented with extra-virgin olive oil (EVOO) or nuts compared with a low-fat control diet (Martinez-Lapiscina et al. 2013a, b). However, MedDiet–gene interactions have not been broadly evaluated in observational studies, and when they were assessed, this assessment only included populations outside of the Mediterranean basin, only evaluated *ApoE4* genotypes, and the results were negative (Cherbuin and Anstey 2012; Roberts et al. 2010). To our knowledge, no previous study has ever assessed gene–diet interactions on cognition in the context of a long-term trial, where subjects were randomly allocated to dietary patterns.

In our study, we aimed to explore the effect of an intervention with MedDiet on cognitive function of participants included in the PREDIMED-NAVARRA randomized trial across different genetic variants not only of *ApoE*, but also of the *CLU, CR1* and *PICALM* genotypes, previously identified as related to cognitive decline through GWAS studies.

Methods

Trial design

The PREDIMED (PREvención con DIeta MEDiterránea) study was a randomized, parallel-group, vascular primary prevention trial conducted in Spain from October 2003 to December 2010 testing two interventions with MedDiet

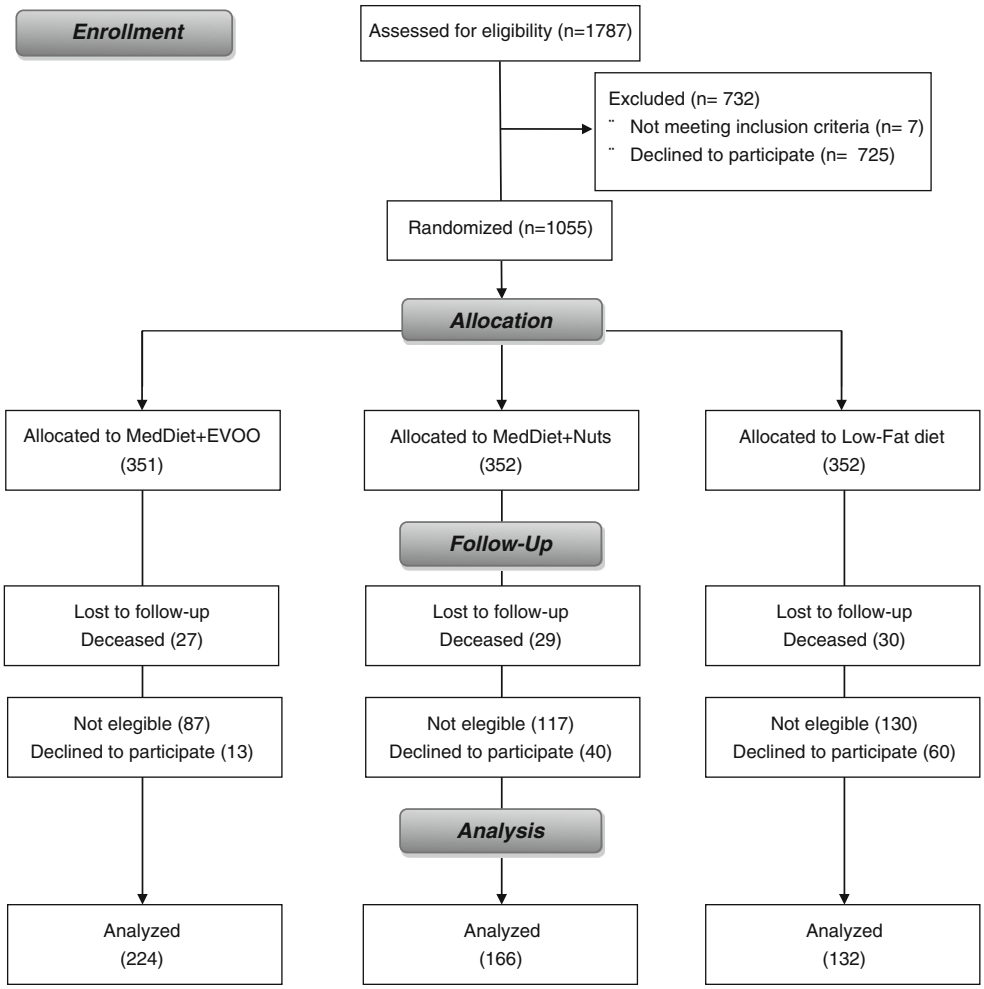

Fig. 1 Flowchart of participants

(supplemented with EVOO or with mixed nuts) versus a control group advised to follow low-fat diet for outcomes on cardiovascular disease events in a high-risk population. The design and methods of the PREDIMED trial have been described in detail elsewhere (Martinez-Gonzalez et al. 2012a). On July 2011, the Data Safety Monitoring Board recommended stopping the trial since the pre-specified stopping boundary for the benefit of the MedDiets on the primary end point had been reached. The primary results have been recently published (Estruch et al. 2013). No relevant diet-related adverse effects were reported (Estruch et al. 2013). The study population was drawn from 1 of 11 recruitment centers (PREDIMED-NAVARRA). At this site, recruitment was completed by 2005, offering thus a longer intervention and follow-up period than other centers.

Study population

Participants were community-dwelling men (55–80 years) and women (60–80 years), without cardiovascular disease (CVD) at baseline but at high vascular risk. The inclusion criteria were the presence of either type-2 diabetes or at least three major risk factors: current smoking, hypertension, dyslipidemia, overweight or family history of premature coronary heart disease. The presence of prior history of CVD, any severe chronic condition or illiteracy was considered as an exclusion criterion (Martinez-Gonzalez et al. 2012a). All procedures followed the Declaration of Helsinki. The Institutional Review Board of the Navarra recruitment center approved the study protocol (protocol 50/2005). All participants signed an informed consent. The PREDIMED-NAVARRA center recruited 1,055 participants between 2003 and 2005.

The cognitive study was conducted over 8 months in the primary care centers in the same day that the nurse performed the blood analyses or the dietitians administered the group or individual sessions for the PREDIMED trial. This routine provides the participants the opportunity to complete different task of the PREDIMED study on the same day and promotes compliance. Participants who did not

attend the visits on their scheduled days were considered non-eligible for the cognitive study. Those who attended the visits but did not accept undergoing the neuropsychological study were excluded. Finally, 522 subjects accepted to participate among 969 participants who were alive at the moment of the cognitive evaluation (Fig. 1).

Nutrition interventions and dietary assessment

A nutritional intervention promoting the MedDiet or advising to follow a lower fat diet was implemented among participants allocated to the two MedDiet groups or control group, respectively. At inclusion and quarterly thereafter, dietitians administrated individual and group sessions, separately for each group to provide an intensive education to follow the intended intervention. Additionally, participants allocated the MedDiet groups received free allotments of either EVOO (1 l/week) or 30 g/day of raw, unprocessed mixed nuts (15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts). Non-food gifts were provided to the control group to improve compliance. Energy restriction was not advised, nor physical activity promoted (Martinez-Gonzalez et al. 2012a; Zazpe et al. 2008).

At baseline and yearly thereafter, a trained dietitian administered a validated 137-item food-frequency questionnaire and a 14-item short questionnaire of adherence to the MedDiet in a face-to-face interview. Both questionnaires have been validated (Fernandez-Ballart et al. 2010; Martinez-Gonzalez et al. 2012b).

Cognitive assessment

Two brief cognitive tests were administered after a mean follow-up of 6.5 years: the Mini-Mental State Examination (MMSE) and the Clock Drawing Test (CDT). MMSE evaluates orientation, registration, calculation, immediate and differed verbal recall, language and visual construction. MMSE ranges from 0 to 30 (Folstein et al. 1975). There is a validated Spanish version (Blesa et al. 2001). The CDT is especially useful for assessing executive function and visuospatial skills that are under-evaluated in the MMSE (Freedman et al. 1994). We used a validated Spanish version ranging from 0 to 7 (del Ser Quijano et al. 2004). Both cognitive tests are valid indicators of cognitive impairment (Crook et al. 1986; Paganini-Hill and Clark 2011). Researchers who assessed the outcome were blinded to group assignment.

Genotyping

CR1, CLU and *PICALM* genotyping was performed for subjects included in the NAVARRA recruitment center of the PREDIMED trial. DNA was extracted from overnight fasting venous blood samples, collected in EDTA added tubes. A commercial kit (Master PureTM; Epicentre, Madison, WI, USA) was used to extract DNA from the buffy coat fraction. All the subjects were genotyped for the rs3818361 of the *CR1* gene, rs11136000 of *CLU* gene and rs3851179 of *PICALM* gene using Taqman with allele-specific probes on the ABI Prism 7300HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to standardized laboratory protocols.

ApoE genotyping was carried out blind at the Genetic and Molecular Epidemiology Unit, Valencia, Spain, for all participants of the PREDIMED trial. Genomic DNA was extracted from buffy coat with the MagNaPure LC DNA Isolation Kit (Roche Diagnostics, Mannheim, Germany). *ApoE* genotyping was carried out by a validated single-tube protocol using fluorescent probes in the LightTyper instrument (Roche), as previously reported (Frances et al. 2008). Quality control procedures including positive and negative controls as well as replication of a random 15 % of samples were applied. The duplicate concordance rate was higher than 98 %.

Covariate assessment

The baseline general questionnaire provided information about socio-demographic, lifestyle features and health-related conditions and family history of cognitive impairment or dementia (Martinez-Gonzalez et al. 2012a). Anthropometric measurements were performed by standard methods, as previously described (Martinez-Gonzalez et al. 2012a). Physical activity was assessed with the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire and expressed in minutes at a given metabolic equivalent per day (MET-min/day) (Elosua et al. 1994, 2000).

Statistical analyses

Hardy–Weinberg equilibrium was tested using a chi-square test. First, we explored differences in baseline characteristics of participants according to the allocated intervention groups. The association between the genotypes and cognitive tests (MMSE and CDT) according to the intervention groups (MedDiet groups vs. control group) were analyzed using linear regression models after adjusting for confounding variables: age (years), sex, education (years of formal education), *ApoE* genotype, family history of cognitive impairment or dementia, hypertension, dyslipidemia, type-2 diabetes mellitus, smoking status, alcohol intake (g/day), body mass index (BMI) (kg/m^2), total energy intake (kcal/day) and physical activity (MET-min/day). Interaction terms were not included in the models. Individual models, one for each SNP (*CLU*, *CR1* and *PICALM*) and

Table 1 Genomic characteristics of the SNP markers used in the analysis

Gene_chr-band	SNPs	bp	Alleles	MA	MAF	*p* for Hardy–Weinberg equilibrium*	OR	95 % CI
CLU_8p21.1	rs11136000	27,520,436	C/T	T	0.4	0.84	0.86	0.81–0.90
CR1_1q32.2	rs3818361	205,758,672	G/A	A	0.2	0.77	1.21	1.14–1.29
PICALM_11q14.2	rs3851179	85,546,288	C/T	T	0.37	0.12	0.86	0.81–0.92

The PREDIMED-NAVARRA cognitive study

SNP single nucleotide polymorphisms, *bp* base pair physical position from the March 2006 human reference sequence (NCBI Build 36.1) produced by the International Human Genome Sequencing Consortium, *MA* allele with the minimum frequency, *MAF* minimum allele frequency, *OR* odd ratios, *95 % CI* 95 % confidence interval

* Chi-square test for testing the Hardy–Weinberg equilibrium in the PREDIMED-NAVARRA study population

for the *ApoE* genotype, were fitted. Interactions between gene variants (dominant models) and the intervention groups (MedDiet groups vs. control group) were estimated with the likelihood ratio test in separated models for each SNPs and adjusted for sex, age, education, *ApoE* genotype (when pertinent), family history of cognitive impairment or dementia and BMI. The false discovery rate method from Benjamini and Hochberg was used to control for multiple testing in the subgroups analysis (Benjamini and Hochberg 1995). Values in the text are means and SDs unless otherwise indicated. All *p* values were two-tailed at the <0.05 level. Statistical analyses were performed with STATA version 12.0 software.

Results

In our population, the frequencies of these three SNPs did fulfill the Hardy–Weinberg equilibrium (Table 1). Anthropometric and lifestyle baseline features of participants of the PREDIMED-NAVARRA study according to the intervention group are displayed in Table 2. As expected from the randomized design, the groups were well balanced with respect to all these baseline characteristics. Since we evaluated the effect of MedDiet as a whole dietary pattern, we have merged participants of MedDiet + EVOO and MedDiet + Nuts groups in a single MedDiet intervention group to compare this intervention fostering adherence to the MedDiet pattern to a low-fat intervention of control group.

As shown in Tables 3 and 4, we did not find statistically significant differences in cognitive function scores (MMSE and CDT) across the considered genetic variants. Nevertheless, the MedDiet intervention seemed to modulate the effect of these genetic variants. In Table 3, the likelihood ratio test showed a statistically significant interaction between the *CLU* gene rs11136000 variant and the intervention status for the MMSE test ($p = 0.041$). For this SNP, we observed a beneficial effect of the MedDiet on MMSE score among subjects carrying the T minor allele (with a previously described protective effect). For these participants, in those carrying at least one copy of the T minor allele, the intervention with the MedDiet was associated with an increase in 0.97 points of the MMSE score ($B = 0.97$, 95 % CI 0.45–1.49, $p < 0.001$). This observation remained statistically significant after the Benjamini–Hochberg multiple comparison analysis. This effect was not observed for those without the T minor allele ($B = 0.18$, 95 % CI -0.52 to 0.88, $p = 0.612$). A similar effect was observed for the *CR1* gene rs3818361 variant, even if in this case, the likelihood ratio test did not show a statistically significant interaction ($p = 0.335$). Within this frame, the MedDiet intervention significantly improved the cognitive function measured by MMSE, 0.76 points, in those subjects without the A minor risk allele ($B = 0.76$, 95 % CI 0.32–1.20, $p = 0.001$). No significant interaction was observed between the *PICALM* gene rs3851179 polymorphisms ($p = 0.872$); however, when stratifying our population according to the dominant model, we observed a beneficial effect of the MedDiet intervention in both genotype groups, in subjects without the T minor allele (protective allele) and in subjects bearing at least one T allele ($B = 0.76$, 95 % CI 0.10–1.43, $p = 0.024$ and $B = 0.51$, 95 % CI 0.01–1.00, $p = 0.046$, respectively). However, this observation did not remain statistically significant after the Benjamini–Hochberg multiple comparison correction.

Table 4 shows similar analyses for the CDT. In this case, no interaction was found between the MedDiet intervention and any of these gene variants. However, differences between genotype groups were found. For the *CLU* gene rs11136000 variant, among subjects with the T minor allele, MedDiet intervention significantly improved CDT scores in 0.60 points ($B = 0.60$, 95 % CI 0.24–0.96, $p = 0.001$). Similar effects were observed for the *CR1* gene rs3818361 variant within subjects without the A risk allele ($B = 0.46$, 95 % CI 0.13–0.79, $p = 0.006$) and for the *PICALM* gene rs3851179 polymorphism. For this SNP,

Table 2 Baseline characteristics of the participants according to intervention group

	MedDiet + EVOO	MedDiet + nuts	Control (low-fat diet)
n	220	161	129
Male (%)	46	42	45
Age at baseline (years)	67 (6)	67 (6)	67 (6)
Hypertension (%)	77.3	82.6	81.4
Dyslipidemia (%)	70.5	69.6	66.7
Diabetes (%)	37.7	36.0	27.1
ApoE4 genotype (%)	13.6	15.1	15.6
CLU (rs11136000) MAF (%)	0.37	0.37	0.39
CR1 (rs3818361) MAF (%)	0.17	0.11	0.14
PICALM (3851179) MAF (%)	0.39	0.30	0.34
Education (years)	8.5 (2.8)	8.4 (2.9)	8.5 (3.4)
Family history of cognitive decline (%)	15.9	13.04	14.7
Body mass index (kg/m^2)	29.3 (3.4)	28.9 (3.2)	29.0 (3.4)
Physical activity (MET-min/day)	283 (199)	279 (196)	252 (198)
Smoking status (%)			
Current smoker	15	12	19
Former smoker	21	22	22
Energy intake (kcal/d)	2,272 (538)	2,263 (490)	2,190 (516)
Alcohol intake (g/day)	14 (21)	13 (18)	11 (18)
MedDiet Score	9.3 (1.8)	9.5 (1.8)	8.6 (1.9)

Data are shown as mean (SD) unless otherwise stated

The PREDIMED-NAVARRA trial

MAF minor allele frequency, *MedDiet Score* Mediterranean diet score

subjects with at least one T minor allele with a probable protective effect on cognition benefited from the MedDiet intervention and their score in the CDT was significantly increased in 0.59 points ($B = 0.59$, 95 % CI 0.18–1.00, $p = 0.005$). All these observations remained statistically significant after the Benjamini–Hochberg multiple comparison correction.

Finally, Table 5 displays the effects of the MedDiet intervention on cognition (MMSE and CDT) in our population according to the *ApoE* genotype. In the first general analysis, the linear regression model revealed that E4 isoform was associated with a significantly decreased score for both cognitive tests, MMSE ($B = -0.96$, 95 % CI -1.47 to -0.45, $p < 0.001$) and CDT ($B = -0.50$, 95 % CI -0.87 to -0.14, $p = 0.007$). Interestingly, when stratifying our subjects accordingly to their genotype for *ApoE4*, we observed that the MedDiet intervention had a protective effect on cognitive function for both non-*ApoE4* and *ApoE4* carriers. For the MMSE score, we found statistically differences in participants allocated to the MedDiet intervention among non-*ApoE4* carriers. The MedDiet intervention was associated with a 0.56-point higher average MMSE score ($B = 0.56$ 95 % CI 0.15–0.97, $p = 0.007$). Specifically, in those carrying the E4 risk isoform, the MedDiet was associated with an increased score in more than 1.6 points ($B = 1.61$, 95 % CI 0.10–3.13, $p = 0.037$). However, this observation did not remain statistically significant after de Benjamini–Hochberg multiple comparison correction. A higher CDT score was also found among subjects randomly allocated to the MedDiet intervention, but this difference was only significant for non-*ApoE4* carriers ($B = 0.55$, 95 % CI 0.25–0.85, $p < 0.001$).

Discussion

In this study, an intervention with a MedDiet modulated the association of genetic risk factors on cognition. The interaction between MedDiet and the *CLU* gene rs11136000 variant is a novel finding. Overall, the protective effect on cognition of an intervention with MedDiet was greater in participants with a favorable genetic profile of the *CR1* gene (without A minor allele of the rs3818361); the *CLU* gene (with T minor allele of the rs11136000); and the *PICALM* gene (with T minor allele of the rs3851179). The only exception was the higher CDT score found in participants without the T minor protective allele of the rs3851179 polymorphism of the *PICALM* gene compared to those with the T minor allele of this polymorphism. Considering the *ApoE* genotype, a MedDiet intervention benefited both non-*ApoE4* and *ApoE4* carriers. Our results strongly suggest a MedDiet effect on both MMSE and CDT assessments among *non-ApoE4* carriers, but the relatively low number of *ApoE4* carriers in our study hinders definite conclusions regarding this group.

For more than 20 years, it has been known that *ApoE4* genotype is a genetic risk factor for Alzheimer's disease

Table 3 Multivariable-adjusted means and differences (95 % CI) for the association between each of the SNPs and cognitive assessment (Mini-Mental State Examination) according to intervention status

Overall	*CLU* (rs11136000) CC ($n = 198$)		*CLU* (rs11136000) CT/TT ($n = 309$)	
Means (95 % CI)	27.72 (27.44 to 28.00)		27.93 (27.70 to 28.15)	
Adj. diff.	0 (Ref.)		+0.21 (−0.16 to +0.57)	
p value			0.266	
By intervention	Control ($n = 45$)	MedDiet[c] ($n = 153$)	Control ($n = 83$)	MedDiet[c] ($n = 226$)
Means (95 % CI)	27.56 (26.95 to 28.16)	27.74 (27.42 to 28.06)	27.23 (26.80 to 27.68)	28.20 (27.94 to 28.50)
B, Adj. diff. (95 % CI)[a]	0 (Ref.)	0.18 (−0.52 to 0.88)	0 (Ref.)	0.97 (0.45 to 1.49)
p value (MedDiet vs. control)		0.612		<0.001[d]
p for interaction[b]	0.041			
Overall	*CR1* (rs3818361) GG ($n = 375$)		*CR1* (rs3818361) GA/AA ($n = 133$)	
Means (95 % CI)	27.88 (27.68 to 28.09)		27.75 (27.40 to 28.09)	
Adj. differences	0 (Ref.)		−0.13 (−0.54 to 0.27)	
p value			0.516	
By intervention	Control ($n = 97$)	MedDiet[c] ($n = 278$)	Control ($n = 32$)	MedDiet[c] ($n = 101$)
Means (95 % CI)	27.37 (26.99 to 27.75)	28.12 (27.90 to 28.34)	27.51 (26.68 to 28.34)	27.66 (27.21 to 28.12)
B, Adj. diff. (95 % CI)[a]	0 (Ref.)	0.76 (0.32 to 1.20)	0 (Ref.)	0.15 (−0.83 to 1.13)
p value (MedDiet vs. control)		0.001[d]		0.762
p for interaction[b]	0.335			
Overall	*PICALM* (rs3851179) CC ($n = 256$)		*PICALM* (rs3851179) CT/TT ($n = 248$)	
Means (95 % CI)	27.70 (27.45 to 27.95)		27.98 (27.73 to 28.23)	
Adj. differences	0 (Ref.)		0.28 (−0.08 to 0.64)	
p value			0.128	
By intervention	Control ($n = 60$)	MedDiet[c] ($n = 196$)	Control ($n = 69$)	MedDiet[c] ($n = 179$)
Means (95 % CI)	27.18 (26.61 to 27.75)	27.89 (27.58 to 28.20)	27.61 (27.19 to 28.03)	28.11 (27.86 to 28.37)
B, Adj. diff. (95 % CI)[a]	0 (Ref.)	0.76 (0.10 to 1.43)	0 (Ref.)	0.51 (0.01 to 1.00)
p value (MedDiet vs. control)		0.024		0.046
p for interaction[b]	0.872			

[a] General linear models adjusted for sex, age, education, family history of cognitive impairment or dementia, *ApoE* genotype, hypertension, dyslipidemia, diabetes, smoking status, alcohol intake, body mass index, physical activity and total energy intake. B represents the difference in points with the reference group

[b] Likelihood ratio test: SNP (dominant model)*intervention status, adjusted for sex, age, education, *ApoE* genotype, family history of cognitive decline and BMI (kg/m^2)

[c] Intervention group gathers MedDiet intervention groups, MedDiet + EVOO (extra-virgin olive oil) and MedDiet + Nuts as it has been stated in the methods

[d] p value < 0.05 after Benjamini–Hochberg multiple comparison adjustment

(Burke and Roses 1991). Years later, GWAS studies identified variants at other genes such as *CLU, PICALM* and *CR1* as risk factors for Alzheimer's disease (Lambert et al. 2009; Harold et al. 2009). These results have been confirmed in a meta-analysis (Jun et al. 2010). More recently, some of these risk gene variants have been associated with cognitive decline, more concretely with poor memory performance in population-based studies (Barral et al. 2012; Pedraza et al. 2013). Parallel with these studies, there is accruing evidence about the benefits in cognition of lifestyle-related habits such as physical and cognitive and social activities (Hamer and Chida 2009;

Table 4 Multivariable-adjusted means and differences (95 % CI) for the association between each of the SNPs and cognitive assessment (Clock Drawing Test) according to intervention status

Overall		*CLU* (rs11136000) CC ($n = 198$)		*CLU* (rs11136000) CT/TT ($n = 309$)
Means (95 % CI)		5.18 (4.98 to 5.38)		5.33 (5.17 to 5.49)
Adj. diff.		0 (Ref.)		0.15 (−0.11 to 0.41)
p value				0.265
By intervention	Control ($n = 45$)	MedDiet[c] ($n = 153$)	Control ($n = 83$)	MedDiet[c] ($n = 226$)
Means (95 % CI)	4.83 (4.39 to 5.21)	5.24 (5.01 to 5.48)	4.90 (4.60 to 5.21)	5.50 (5.32 to 5.69)
B, Adj. diff. (95 % CI)[a]	0 (Ref.)	0.40 (−0.11 to 0.92)	0 (Ref.)	0.60 (0.24 to 0.96)
p value (MedDiet vs. control)		0.126		0.001[d]
p for interaction[b]	0.366			
Overall		*CR1* (rs3818361) GG ($n = 375$)		*CR1* (rs3818361) GA/AA ($n = 133$)
Means (95 % CI)		5.28 (5.13 to 5.42)		5.26 (5.01 to 5.50)
Adj. differences		0 (Ref.)		−0.02 (−0.30 to 0.27)
p value				0.900
By intervention	Control ($n = 97$)	MedDiet[c] ($n = 278$)	Control ($n = 32$)	MedDiet[c] ($n = 101$)
Means (95 % CI)	4.96 (4.68 to 5.25)	5.43 (5.26 to 5.59)	4.92 (4.40 to 5.45)	5.24 (4.95 to 5.53)
B, Adj. diff. (95 % CI)[a]	0 (Ref.)	0.46 (0.13 to 0.79)	0 (Ref.)	0.31 (−0.30 to 0.93)
p value (MedDiet vs. control)		0.006[d]		0.762
p for interaction[b]	0.841			
Overall		*PICALM* (rs3851179) CC ($n = 256$)		*PICALM* (rs3851179) CT/TT ($n = 248$)
Means (95 % CI)		5.35 (5.17 to 5.52)		5.21 (5.03 to 5.39)
Adj. differences		0 (Ref.)		−0.14 (−0.39 to 0.11)
p value				0.275
By intervention	Control ($n = 60$)	MedDiet[c] ($n = 196$)	Control ($n = 69$)	MedDiet[c] ($n = 179$)
Means (95 % CI)	5.19 (4.84 to 5.54)	5.41 (5.22 to 5.60)	4.78 (4.43 to 5.13)	5.36 (5.15 to 5.58)
B, Adj. diff. (95 % CI)[a]	0 (Ref.)	0.27 (−0.13 to 0.68)	0 (Ref.)	0.59 (0.18 to 1.00)
p value (MedDiet vs. control)		0.186		0.005[d]
p for interaction[b]	0.133			

[a] General linear models adjusted for sex, age, education, family history of cognitive impairment or dementia, *ApoE* genotype, hypertension, dyslipidemia, diabetes, smoking status, alcohol intake, body mass index, physical activity and total energy intake. B represents the difference in points with the reference group

[b] Likelihood ratio test: SNP (dominant model)*intervention status, adjusted for sex, age, education, *ApoE* genotype, family history of cognitive decline and BMI (kg/m^2)

[c] Intervention group gathers MedDiet intervention groups, MedDiet + EVOO (extra-virgin olive oil) and MedDiet + Nuts as it has been stated in the methods

[d] p value < 0.05 after Benjamini–Hochberg multiple comparison adjustment

Kareholt et al. 2011; Morgan et al. 2012; Niti et al. 2008; Sattler et al. 2012) and nutrition in terms of nutrients and foods with conflicting results (Coley et al. 2008). Regarding dietary patterns as a whole, a protective effect of MedDiet on cognitive function (Kesse-Guyot et al. 2013), cognitive decline (Tsivgoulis et al. 2013; Tangney et al. 2011; Feart et al. 2009; Scarmeas et al. 2006) and development of mild cognitive impairment (Scarmeas et al. 2009b; Roberts et al. 2010) and dementia (Roberts et al. 2010; Gu et al. 2010; Scarmeas et al. 2006, 2009a, b) has been found in several observational studies but not in other cohort studies (Psaltopoulou et al. 2008; Samieri et al.

Table 5 Multivariable-adjusted means and differences (95 % CI) for the association between the SNPs and cognitive assessment according to intervention status

	Mini-Mental State Examination			Clock Drawing Test		
	B (95 % CI)[a]	*p* value	*p* for interaction[b]	B (95 % CI)[a]	*p* value	*p* for interaction[b]
APOE			0.665			0.091
E2 carriers (32)	0.44 (−0.29 to 1.17)	0.241		0.25 (−0.27 to 0.77)	0.348	
E3/E3 (401)	0 (Ref.)			0 (Ref.)		
E4 carriers (74)	−0.96 (−1.47 to −0.45)	<0.001[d]		−0.50 (−0.87 to −0.14)	0.007[d]	
Non-E4 carriers						
Control (Low-fat diet) (108)	0 (Ref.)			0 (Ref.)		
Intervention (MedDiet[c]) (325)	0.56 (0.15 to 0.97)	0.007[d]		0.55 (0.25 to 0.85)	<0.001[d]	
E4 carriers						
Control (Low-fat diet) (20)	0 (Ref.)			0 (Ref.)		
Intervention (MedDiet[c]) (54)	1.61 (0.10 to 3.13)	0.037		0.33 (−0.60 to 1.27)	0.477	

The numbers in parentheses are the subjects with this genotype

MMSE Mini-Mental State Examination, *CDT* Clock Drawing Test, *Ref.* Advert this is the reference group

[a] General linear models adjusted for sex, age, education, family history of cognitive impairment or dementia, hypertension, dyslipidemia, diabetes, smoking status, alcohol intake, body mass index, physical activity and total energy intake. B represents the difference in points with the reference group

[b] Likelihood ratio test: SNP (dominant model)*intervention status, adjusted for sex, age, education, *ApoE* genotype, family history of cognitive decline and BMI (kg/m^2)

[c] Intervention group gathers MedDiet intervention groups, MedDiet + EVOO (extra-virgin olive oil) and MedDiet + Nuts as it has been stated in the methods

[d] p value < 0.05 after Benjamini–Hochberg multiple comparison adjustment

2013; Cherbuin and Anstey 2012). Thus, there is not definitive evidence from observational studies to ensure the benefits of an intervention with MedDiet against cognitive impairment. However, a protective effect has been recently suggested by the PREDIMED-NAVARRA randomized trial conducted in Spain (Martinez-Lapiscina et al. 2013a, b). This is important since some observational studies may not find a protective association between the MedDiet and cognitive function because their MedDiet-like dietary pattern did not truly represent the traditional MedDiet due to sociocultural factors (Cherbuin and Anstey 2012). Nevertheless, the benefit of MedDiet on cognitive impairment is far from being resolved. Going a step beyond, the interaction between MedDiet and risk factors has been poorly studied. In a study conducted in Australia, the MedDiet did not show any protective association with cognitive changes and no significant interaction was observed for the *ApoE4* genotype and the MedDiet (Cherbuin and Anstey 2012). We did not identify any studies assessing the interaction between MedDiet and *CR1, CLU* and *PICALM* gene variants.

We acknowledge that our study may have some limitations. First, cognitive function of participants was not assessed at baseline. Nevertheless, the presence of any chronic condition that may limit the compliance with the protocol was an exclusion criterion, and this is a good reason to confide that a relevant cognitive decline was not present at baseline. In addition, the randomized design of the trial resulted in well-balanced groups with respect to the overall baseline features, and this argues for similar cognitive status at baseline. Moreover, we performed our analyses after adjusting for a wide range of potential confounders. Second, the sample size was small. Third, the study has a single blinded nature, but there is no possibility of conducting true double-blind long-term trials in nutrition. The selection of a low-fat dietary pattern as comparator might have introduced a limitation in our results, because of the potential protection afforded by educating the control group on a low-fat diet. The role of this dietary pattern on cognition is not completely understood. Should the low-fat diet have a detrimental effect, the allocation to a low-fat diet for comparison in the control group may represent an alternative explanation to the protective effect observed for the MedDiet. However, the long-term sustainability of low-fat diet is suboptimal, and in any case, controls may have experienced a benefit from their education on a low-fat diet that would have not existed should they have followed a Western-type diet. Another limitation is that we evaluated the interaction between MedDiet and gene risk variants in a

high-risk vascular population so generalization of our findings to the average general population is uncertain. Finally, we acknowledge that our results are relevant in terms of statistical significance, but they do not represent a large difference from the clinical or practical point of view. However, we think that our manuscript is scientifically relevant because it points to a biological interaction between MedDiet and gene profile beyond ApoE. The introduction of this interaction is a novelty, and our exploratory results may favor further and deeper studies of gene–nutrition interactions in larger cohorts that may consider not only cognitive function but also, more importantly, the incidence of clinical cases of mild cognitive impairment or dementia.

Our study has also strengths. First, this is a long-term randomized controlled trial with supplementation with hallmark components of the traditional MedDiet such as EVOO and nuts. Second, we included several potential confounders in our analyses that help us to rule out residual confounding.

In conclusion, we provide here, for the first time, the evidence of an interaction between the MedDiet and *CLU* on cognitive function in the context of a randomized trial. A MedDiet intervention modulates the effect of the genetic risk factors on cognition, and overall, the protective effect of MedDiet may be greater for subjects with favorable genetic profile. Future research is needed to obtain firmed evidence of the complex MedDiet–gene interaction.

Acknowledgments The authors thank the participants for their enthusiastic collaboration, the PREDIMED personnel for excellent assistance and the personnel of all affiliated primary care centers. Fundación Patrimonio Comunal Olivarero and Hojiblanca SA, California Walnut Commission, Borges SA, and Morella Nuts SA generously donated the olive oil, walnuts, almonds and hazelnuts, respectively, used in the study. None of the mentioned food companies played or will play any role in the design, collection, analysis or interpretation of the data or in the decision to submit manuscripts for publication. This study was funded by the official agency for funding biomedical research of the Spanish Government (ISCIII) through the following competitive grants: PI1001407, PI1002293 PI081943, PI070240, PI050976, Thematic Network Nutrition & Cardiovascular disease G03/140 (Coordinator Ramón Estruch), Thematic Network PREDIMED RD06/0045 (Coordinator Miguel A. Martínez-Gonzalez), FEDER (Fondo Europeo de Desarrollo Regional) and CIBERobn (Virtual Center for Biomedical Research on Obesity and Nutrition). CIBERobn is an initiative of ISCIII, Spain. Additional funding was provided by the Government of Navarra (PI41/2005, PI36/2008, PI27/2012). None of the funding sources played a role in the design, collection, analysis or interpretation of the data or in the decision to submit the manuscript for publication. E. Toledo is supported by a Rio Hortega post-residency fellowship of ISCIII, Ministry of Economy and Competitiveness, Spanish Government.

Conflict of interest Dr. Martínez-Lapiscina has received travel and accommodation expenses from Novartis, Biogen, Teva and Bayer for national and international congress. Dr. Salas-Salvadó reports serving on the board of and receiving grant support through his institution from the International Nut and Dried Fruit Council; reports serving on the board of Instituto Danone (Spain); receiving consulting fees from Danone; and receiving grant support through his institution from Feiraco, Eroski and Nestlé. Dr. Ros is a non paid member of the Scientific Advisory Committee of the California Walnut Commission. He has received research funding (grants) from AMGEN, California Walnut Commission, KARO-BIO, Merck, Sanofi Aventis, and Synageva. He has receveid honoraria for educational conferences from Aegerion, Alter, Astra Zeneka, DANONE, Ferrer International, Merck, Progenika, Roche, and Rottapharm. ER has also received payment for educational presentations for Abbott, Ferrer International, and Ricordati. He has received travel and accommodation expenses from California Walnut Commission and International Nut Council for attending and delivering speeches at international congresses. Dr. Galbete, Dr. Toledo, Dr. Corella, Dr. Buil-Cosiales and Dr. Martinez-Gonzalez declare that they have no conflict of interest.

Appendix: PREDIMED Group collaborators

University of Navarra and Osasunbidea (Servicio Navarro de Salud), Primary Care Centres, Pamplona, Spain: M. Ruiz-Canela, B. Sanjulian, A. Sanchez-Tainta, E. Goñi, A. Alonso, C. Razquin, Z. Vzquez, M Marques, I. Zazpe, A. Marti, J. Diez-Espino, A. Garcia-Arellano, J. Basterra-Gortari, V. Extremera-Urabayen, C. Arroyo-Azpa, LGarcia-Perez, J. Villanueva Telleria, F. Cortes Ugalde, T. Sagredo Arce, M. D.Garcia de la Noceda Montoy, M. D. Vigata Lopez, M. T. Arceiz Campo, A. Urtasun Samper, M. V. Gueto Rubio, and B. Churio Beraza.

Hospital Clinic, Institut d'Investigacions Biomediques August Pi i Sunyer, Barcelona, Spain: R Estruch, M. Serra, A. Perez-Heras, C. Vinas, R. Casas, L. de Santamaria, S. Romero, J.M. Baena, M. Garcia, M. Oller, J. Amat, I. Duaso, Y. Garcia, C. Iglesias, C. Simon, Ll. Quinzavos, Ll. Parra, M. Liroz, J. Benavent, J. Clos, I. Pla, M. Amoros, M.T. Bonet, M.T. Martin, M.S. Sanchez, J. Altirruba, E. Manzano, A. Altes, M. Cofan, C. Valls-Pedret, A. Sala-Vila, and M. Domenech.

University Rovira i Virgili, Reus, Spain: M. Bullo, J. Basora, R. Gonzalez, C. Molina, F. Marquez, N. Babio, P, Martinez, N. Ibarrola, R. Balanza, M. Sorli, J. Garcia Roselló, F. Martin, R. Tort, A. Isach, B. Costa, J.J. Cabre, and J. Fernandez-Ballart.

University of Valencia, Valencia, Spain: J.V. Sorli, P. Carrasco, R. Osma, M. Guillen, P. Guillem-Saiz, O. Portoles, V. Pascual, C. Riera, J. Valderrama, A. Serrano, E. Lazaro, A. Sanmartin, A. Girbes, V. Santamaria, C. Sanchez and Z. Pla.

University Hospital of Alava, Vitoria, Spain: F. Aros, I. Salaverria, T. del Hierro, J. Algorta, S. Francisco, A. Alonso, J. San Vicente, A. Casi, E. Sanz, I. Felipe, J. Rekondo and A. Loma-Osorio.

University of Malaga, Malaga, Spain: E. Gomez-Gracia, J. Wärnberg, R. Benitez Pont, M. Bianchi Alba, J. Fernandez-Crehuet Navajas, J. Warnberg, R. Gomez-Huelgas,

J. Martinez-Gonzalez, V. Velasco Garcia, J. de Diego Salas, A. Baca Osorio, J. Gil Zarzosa, J.J. Sanchez Luque, and E. Vargas Lopez.

Institute de Recerca Hospital del Mar, Barcelona, Spain: M. Fito, M.I. Covas, S. Tello, J. Vila, R. de la Torre, D. Munoz-Aguayo, R. Elosua, J. Marrugat, and M. Ferrer.

Instituto de la Grasa, Consejo Superior de Investigaciones Cientificas, Sevilla, Spain: V. Ruiz-Gutierrez, J. Sanchez Perona, E. Montero Romero, M. Garcia–Garcia and E. Jurado-Ruiz.

Institute of Health Sciences IUNICS, University of Balearic Islands, and Hospital Son Espases, Palma de Mallorca, Spain: M. Fiol, M. Garcia-Valdueza, M. Monino, A. Proenza, R. Prieto, G. Frontera, M. Ginard, F. Fiol, A. Jover, D. Romaguera and J. Garcia.

Department of Family Medicine, Primary Care Division of Sevilla, Sevilla, Spain: J. Lapetra, M. Leal, E. Martinez, J.M. Santos, M. Ortega-Calvo, P. Roman, F. Jose Garcia, P. Iglesias, Y. Corchado, E. Mayoral, L. Mellado, L. Miró, JM. Lozano and C. Lama.

School of Pharmacy, University of Barcelona, Barcelona, Spain: R.M. Lamuela-Raventos, M.C. Lopez-Sabater, A.I. Castellote-Bargallo, A. Medina-Remon, and A. Tresserra-Rimbau.

University of Las Palmas de Gran Canaria, Las Palmas, Spain: L. Serra-Majem, A. Sanchez-Villegas,J. Alvarez-Perez, E. Diez Benitez, I. Bautista Castaño, I. Maldonado Diaz, M.J. Férnandez-Rodríguez, F. Sarmiendo de la Fe, C. Simon Garcia, I. Falcon Sanabria, B. Macias Gutierrez, and A.J. Santana Santana.

Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain: X. Pinto, E. de la Cruz, A. Galera, Y. Soler, F. Trias, I. Sarasa, E. Padres, and E. Corbella.

Primary Care Division, Catalan Institute of Health, Barcelona, Spain: M. Muñoz, C. Cabezas, E. Vinyoles, M.A. Rovira, L. Garcia, G. Flores, J.M. Verdu, P. Baby, A. Ramos, L. Mengual, P. Roura, M.C. Yuste, A. Guarner, A. Rovira, M.I. Santamaria, M. Mata, C. de Juan, and A. Brau.

Other investigators of the PREDIMED network: J.A. Martinez (University of Navarra), J.A. Tur (University of Balearic Islands), M.P. Portillo (University of Basque Country) and G. Saez (University of Valencia).

References

Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, Alperovitch A (2007) Dietary patterns and risk of dementia: the three-city cohort study. Neurology 69(20):1921–1930. doi:10.1212/01.wnl.0000278116.37320.52

Barral S, Bird T, Goate A, Farlow MR, Diaz-Arrastia R, Bennett DA, Graff-Radford N, Boeve BF, Sweet RA, Stern Y, Wilson RS, Foroud T, Ott J, Mayeux R (2012) Genotype patterns at PICALM, CR1, BIN1, CLU, and APOE genes are associated with episodic memory. Neurology 78(19):1464–1471. doi:10.1212/WNL.0b013e3182553c48

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300

Birks J (2006) Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev (1):CD005593. doi:10.1002/14651858.cd005593

Blesa R, Pujol M, Aguilar M, Santacruz P, Bertran-Serra I, Hernandez G, Sol JM, Pena-Casanova J (2001) Clinical validity of the 'mini-mental state' for Spanish speaking communities. Neuropsychologia 39(11):1150–1157

Burke JR, Roses AD (1991) Genetics of Alzheimer's disease. Int J Neurol 25–26:41–51

Chang-Quan H, Hui W, Chao-Min W, Zheng-Rong W, Jun-Wen G, Yong-Hong L, Yan-You L, Qing-Xiu L (2011) The association of antihypertensive medication use with risk of cognitive decline and dementia: a meta-analysis of longitudinal studies. Int J Clin Pract 65(12):1295–1305. doi:10.1111/j.1742-1241.2011.02810.x

Cherbuin N, Anstey KJ (2012) The Mediterranean diet is not related to cognitive change in a large prospective investigation: the PATH Through Life study. Am J Geriatr Psychiatry 20(7):635–639. doi:10.1097/JGP.0b013e31823032a9

Coley N, Andrieu S, Gardette V, Gillette-Guyonnet S, Sanz C, Vellas B, Grand A (2008) Dementia prevention: methodological explanations for inconsistent results. Epidemiol Rev 30:35–66. doi:10.1093/epirev/mxn010

Crook TBR, Ferris SH et al (1986) Age-associated memory impairment: proposed diagnostic criteria and measures of clinical change—report of a National Institute of Mental Health Work Group. Dev Psychol 2:261–276

del Ser Quijano T, Garcia de Yebenes MJ, Sanchez SF, Frades PB, Rodriguez LA, Bartolome MMP, Otero PA (2004) Cognitive assessment in the elderly. Normative data of a Spanish population sample older than 70 years. Med Clin (Barc) 122(19):727–740

Elosua R, Marrugat J, Molina L, Pons S, Pujol E (1994) Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM investigators. Am J Epidemiol 139(12):1197–1209

Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J (2000) Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc 32(8):1431–1437

Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pinto X, Basora J, Munoz MA, Sorli JV, Martinez JA, Martinez-Gonzalez MA (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 368(14):1279–1290. doi:10.1056/NEJMoa1200303

Exalto LG, Whitmer RA, Kappele LJ, Biessels GJ (2012) An update on type 2 diabetes, vascular dementia and Alzheimer's disease. Exp Gerontol 47(11):858–864. doi:10.1016/j.exger.2012.07.014

Feart C, Samieri C, Rondeau V, Amieva H, Portet F, Dartigues JF, Scarmeas N, Barberger-Gateau P (2009) Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 302(6):638–648. doi:10.1001/jama.2009.1146

Fernandez-Ballart JD, Pinol JL, Zazpe I, Corella D, Carrasco P, Toledo E, Perez-Bauer M, Martinez-Gonzalez MA, Salas-Salvado J, Martin-Moreno JM (2010) Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 103(12):1808–1816. doi:10.1017/s0007114509993837

Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12(3):189–198

Frances F, Portoles O, Sorli JV, Guillen M, Gonzalez JI, Corella D (2008) Single tube optimisation of APOE genotyping based on melting curve analysis. Clin Biochem 41(10–11):923–926. doi:10.1016/j.clinbiochem.2008.03.010

Freedman MLL, Kaplan E et al (1994) Clock drawing. A neuropsychological analysis. Oxford University Press, New York

Gu Y, Luchsinger JA, Stern Y, Scarmeas N (2010) Mediterranean diet, inflammatory and metabolic biomarkers, and risk of Alzheimer's disease. J Alzheimers Dis 22(2):483–492. doi:10.3233/jad-2010-100897

Hamer M, Chida Y (2009) Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. Psychol Med 39(1):3–11. doi:10.1017/s0033291708003681

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schurmann B, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Hull M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41(10):1088–1093. doi:10.1038/ng.440

Hughes TF, Andel R, Small BJ, Borenstein AR, Mortimer JA, Wolk A, Johansson B, Fratiglioni L, Pedersen NL, Gatz M (2010) Midlife fruit and vegetable consumption and risk of dementia in later life in Swedish twins. Am J Geriatr Psychiatry 18(5):413–420. doi:10.1097/JGP.0b013e3181c65250

Jellinger KA, Attems J (2010) Prevalence of dementia disorders in the oldest-old: an autopsy study. Acta Neuropathol 119(4):421–433. doi:10.1007/s00401-010-0654-5

Jun G, Naj AC, Beecham GW, Wang LS, Buros J, Gallins PJ, Buxbaum JD, Ertekin-Taner N, Fallin MD, Friedland R, Inzelberg R, Kramer P, Rogaeva E, St George-Hyslop P, Cantwell LB, Dombroski BA, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Lunetta KL, Martin ER, Montine TJ, Goate AM, Blacker D, Tsuang DW, Beekly D, Cupples LA, Hakonarson H, Kukull W, Foroud TM, Haines J, Mayeux R, Farrer LA, Pericak-Vance MA, Schellenberg GD (2010) Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol 67(12):1473–1484. doi:10.1001/archneurol.2010.201

Kareholt I, Lennartsson C, Gatz M, Parker MG (2011) Baseline leisure time activity and cognition more than two decades later. Int J Geriatr Psychiatry 26(1):65–74. doi:10.1002/gps.2490

Kesse-Guyot E, Andreeva VA, Lassale C, Ferry M, Jeandel C, Hercberg S, Galan P (2013) Mediterranean diet and cognitive function: a French study. Am J Clin Nutr 97(2):369–376. doi:10.3945/ajcn.112.047993

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fievet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanche H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M, Amouyel P (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41(10):1094–1099. doi:10.1038/ng.439

Liu B, Shen Y, Cen L, Tang Y (2012) Apolipoprotein E gene polymorphism in a Chinese population with vascular dementia: a meta-analysis. Dement Geriatr Cogn Disord 33(2–3):96–103. doi:10.1159/000337025

Mangialasche F, Kivipelto M, Mecocci P, Rizzuto D, Palmer K, Winblad B, Fratiglioni L (2010) High plasma levels of vitamin E forms and reduced Alzheimer's disease risk in advanced age. J Alzheimers Dis 20(4):1029–1037. doi:10.3233/jad-2010-091450

Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ros E, Covas MI, Fiol M, Warnberg J, Aros F, Ruiz-Gutierrez V, Lamuela-Raventos RM, Lapetra J, Munoz MA, Martinez JA, Saez G, Serra-Majem L, Pinto X, Mitjavila MT, Tur JA, Portillo MP, Estruch R (2012a) Cohort profile: design and methods of the PREDIMED study. Int J Epidemiol 41(2):377–385. doi:10.1093/ije/dyq250

Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, Covas MI, Schroder H, Aros F, Gomez-Gracia E, Fiol M, Ruiz-Gutierrez V, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pinto X, Munoz MA, Warnberg J, Ros E, Estruch R (2012b) A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS ONE 7(8):e43134. doi:10.1371/journal.pone.0043134

Martinez-Lapiscina EH, Clavero P, Toledo E, Estruch R, Salas-Salvado J, San Julian B, Sanchez-Tainta A, Ros E, Valls-Pedret C, Martinez-Gonzalez MA (2013a) Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. J Neurol Neurosurg Psychiatry. doi:10.1136/jnnp-2012-304792

Martinez-Lapiscina EH, Clavero P, Toledo E, San Julian B, Sanchez-Tainta A, Corella D, Lamuela-Raventos RM, Martinez JA, Martinez-Gonzalez MA (2013b) Virgin olive oil supplementation and long-term cognition: the predimed-navarra randomized, trial. J Nutr Health Aging 17(6):544–552. doi:10.1007/s12603-013-0027-6

McShane R, Areosa Sastre A, Minakaran N (2006) Memantine for dementia. Cochrane Database Syst Rev (2):CD003154. doi:10.1002/14651858.CD003154.pub5

Morgan GS, Gallacher J, Bayer A, Fish M, Ebrahim S, Ben-Shlomo Y (2012) Physical activity in middle-age and dementia in later life: findings from a prospective cohort of men in Caerphilly, South Wales and a meta-analysis. J Alzheimers Dis 31(3):569–580. doi:10.3233/jad-2012-112171

Niti M, Yap KB, Kua EH, Tan CH, Ng TP (2008) Physical, social and productive leisure activities, cognitive decline and interaction with APOE-epsilon 4 genotype in Chinese older adults. Int Psychogeriatr 20(2):237–251. doi:10.1017/s1041610207006655

Paganini-Hill A, Clark LJ (2011) Longitudinal assessment of cognitive function by clock drawing in older adults. Dement Geriatr Cogn Dis Extra 1(1):75–83. doi:10.1159/000326781

Pedraza O, Allen M, Jennette K, Carrasquillo M, Crook J, Serie D, Pankratz VS, Palusak R, Nguyen T, Malphrus K, Ma L, Bisceglio G, Roberts RO, Lucas JA, Ivnik RJ, Smith GE, Graff-Radford NR, Petersen RC, Younkin SG, Ertekin-Taner N (2013) Evaluation of memory endophenotypes for association with CLU, CR1, and PICALM variants in black and white subjects. Alzheimer's Dement J Alzheimer's Assoc. doi:10.1016/j.jalz.2013.01.016

Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP (2013) The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement 9(1):63–75 e62. doi:10.1016/j.jalz.2012.11.007

Psaltopoulou T, Kyrozis A, Stathopoulos P, Trichopoulos D, Vassilopoulos D, Trichopoulou A (2008) Diet, physical activity and cognitive impairment among elders: the EPIC-Greece cohort (European Prospective Investigation into Cancer and Nutrition). Public Health Nutr 11(10):1054–1062. doi:10.1017/s1368980007001607

Roberts RO, Geda YE, Cerhan JR, Knopman DS, Cha RH, Christianson TJ, Pankratz VS, Ivnik RJ, Boeve BF, O'Connor HM, Petersen RC (2010) Vegetables, unsaturated fats, moderate alcohol intake, and mild cognitive impairment. Dement Geriatr Cogn Disord 29(5):413–423. doi:10.1159/000305099

Roman B, Carta L, Martinez-Gonzalez MA, Serra-Majem L (2008) Effectiveness of the Mediterranean diet in the elderly. Clin Interv Aging 3(1):97–109

Samieri C, Okereke OI, Devore EE, Grodstein F (2013) Long-term adherence to the Mediterranean diet is associated with overall cognitive status, but not cognitive decline, in women. J Nutr. doi:10.3945/jn.112.169896

Sattler C, Toro P, Schonknecht P, Schroder J (2012) Cognitive activity, education and socioeconomic status as preventive factors for mild cognitive impairment and Alzheimer's disease. Psychiatry Res 196(1):90–95. doi:10.1016/j.psychres.2011.11.012

Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA (2006) Mediterranean diet and risk for Alzheimer's disease. Annals of neurology 59(6):912–921. doi:10.1002/ana.20854

Scarmeas N, Luchsinger JA, Schupf N, Brickman AM, Cosentino S, Tang MX, Stern Y (2009a) Physical activity, diet, and risk of Alzheimer disease. JAMA 302(6):627–637. doi:10.1001/jama.2009.1144

Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA (2009b) Mediterranean diet and mild cognitive impairment. Arch Neurol 66(2):216–225. doi:10.1001/archneurol.2008.536

Tangney CC, Tang Y, Evans DA, Morris MC (2009) Biochemical indicators of vitamin B12 and folate insufficiency and cognitive decline. Neurology 72(4):361–367. doi:10.1212/01.wnl.0000341272.48617.b0

Tangney CC, Kwasny MJ, Li H, Wilson RS, Evans DA, Morris MC (2011) Adherence to a Mediterranean-type dietary pattern and cognitive decline in a community population. Am J Clin Nutr 93(3):601–607. doi:10.3945/ajcn.110.007369

Tsivgoulis G, Judd S, Letter AJ, Alexandrov AV, Howard G, Nahab F, Unverzagt FW, Moy C, Howard VJ, Kissela B, Wadley VG (2013) Adherence to a Mediterranean diet and risk of incident cognitive impairment. Neurology 80(18):1684–1692. doi:10.1212/WNL.0b013e3182904f69

van Gelder BM, Tijhuis M, Kalmijn S, Kromhout D (2007) Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. Am J Clin Nutr 85(4):1142–1147

Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D (1995) Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 61(6 Suppl):1402S–1406S

Yin YW, Li JC, Wang JZ, Li BH, Pi Y, Yang QW, Fang CQ, Gao CY, Zhang LL (2012) Association between apolipoprotein E gene polymorphism and the risk of vascular dementia: a meta-analysis. Neurosci Lett 514(1):6–11. doi:10.1016/j.neulet.2012.02.031

Zazpe I, Sanchez-Tainta A, Estruch R, Lamuela-Raventos RM, Schroder H, Salas-Salvado J, Corella D, Fiol M, Gomez-Gracia E, Aros F, Ros E, Ruiz-Gutierrez V, Iglesias P, Conde-Herrera M, Martinez-Gonzalez MA (2008) A large randomized individual and group intervention conducted by registered dietitians increased adherence to Mediterranean-type diets: the PREDIMED study. J Am Diet Assoc 108(7):1134–1144. doi:10.1016/j.jada.2008.04.011 discussion 1145