Apolipoprotein E e4 allele affects risk of hyperhomocysteinemia in the elderly[1–3]

Giovanni Ravaglia, Paola Forti, Fabiola Maioli, Martina Chiappelli, Fausta Montesi, Marisa Bianchin, Federico Licastro, and Christopher Patterson

ABSTRACT

Background: Apolipoprotein E (APOE) plays a central role in VLDL metabolism. Both *APOE e4* allele (*APOE4*) and C-reactive protein (CRP) are associated with greater risk of dementia and vascular disease, but *APOE4* carriers have lower blood concentrations of CRP than do noncarriers, possibly through a mechanism favoring the clearance of the CRP VLDL–bound fraction. Homocysteine, another risk factor for vascular disease and dementia, also binds to VLDL in blood. However, the association between *APOE4* and hyperhomocysteinemia has never been thoroughly investigated.

Objective: We investigated in an elderly population whether *1*) *APOE4* is associated with hyperhomocysteinemia [plasma total homocysteine (tHcy) $>$ 15 μmol/L], *2*) hyperhomocysteinemia affects the association between *APOE4* and high CRP (serum CRP $>$ 3 mg/L), and *3*) B vitamin status affects these associations.

Design: *APOE4* genotypes were assessed and tHcy, CRP, and serum concentrations of folate and vitamin B-12 were measured in 671 cognitively healthy subjects (52% women; mean age: 73 y) from an Italian population–based prospective cohort study.

Results: *APOE4* carriers without high CRP [multivariate-adjusted odds ratio (OR): 0.22; 95% CI: 0.08, 0.59] had a lower risk of hyperhomocysteinemia than did noncarriers. The risk of high CRP was lower in *APOE4* carriers without hyperhomocysteinemia (multivariate-adjusted OR: 0.51; 95% CI: 0.31, 0.85) than in noncarriers. The associations were not affected by B vitamin status.

Conclusion: Independently from B vitamin status, *APOE4* carriers have a lower risk of hyperhomocysteinemia and of high CRP than do noncarriers, but the presence of one condition attenuates the association of *APOE4* with the other condition. *Am J Clin Nutr* 2006;84:1473–80.

KEY WORDS Homocysteine, C-reactive protein, apolipoprotein E, elderly, lipid metabolism

INTRODUCTION

Apoliproprotein E (APOE) plays a central role in lipid metabolism as a component of VLDL and chylomicrons (1). The *APOE* gene, located at chromosome 19, is polymorphic with 3 common alleles—*e2*, *e3*, and *e4* (2). The *APOE e4* allele (*APOE4*) is a main risk factor for Alzheimer dementia and is also associated with vascular dementia and ischemic heart disease (2, 3). With respect to noncarriers, *APOE4* carriers have lower blood concentrations of the inflammatory marker C-reactive protein (CRP) (4–7), which is also a predictor of both vascular events (8) and dementia (9, 10). The ability of circulating CRP to bind to APOE (11) and VLDL (12) may explain the association with *APOE4* (5), because carriers of this allele have the lowest APOE concentrations and, in them, VLDL is removed from the blood with particular efficiency (2).

Elevated blood concentrations of the sulfur amino acid homocysteine are another emerging risk factor for dementia (13, 14) and a predictor of vascular risk (15). Moderate hyperhomocysteinemia is common in elderly people and is usually caused by poor B vitamin status and reduced renal function (16, 17). An important fraction of plasma total homocysteine (tHcy) circulates bound to lipoproteins, particularly to VLDL (18–20), but the possible association of *APOE4* with homocysteinemia has, to our knowledge, never been thoroughly investigated. In this study, we used data from an elderly Italian cohort *1*) to verify the hypothesis that *APOE4* is associated with lower plasma concentrations of tHcy, *2*) to investigate whether hyperhomocysteinemia affects the known association between circulating CRP and *APOE4*, and *3*) to assess the effects of vitamin B status on these associations.

SUBJECTS AND METHODS

Study population

The Conselice Study of Brain Ageing (CSBA) is a population-based prospective survey described in detail elsewhere (21, 22), with the principal aim of exploring the epidemiology of and risk factors for dementia in the elderly. Briefly, in 1999–2000, 1016 (75%) of the 1353 persons aged ≥65 y residing in the Italian municipality of Conselice (the Emilia-Romagna region of Italy) participated in the prevalence study. Participants were screened with the Italian version of the Mini Mental State Examination

[1] From the Department of Internal Medicine, Cardioangiology, and Hepatology, University Hospital S Orsola-Malpighi, Bologna, Italy (GR, PF, FMa, and FMo); the Department of Experimental Pathology, University of Bologna, Italy (MC and FL); the District Health Unit of Lugo, Local Health Unit of Ravenna, Ravenna, Italy (MB); and the Department of Medicine, McMaster University, Hamilton, Canada (CP).

[2] Supported by a Basic Oriented Research grant from the University of Bologna.

[3] Address reprint requests to G Ravaglia, Department of Internal Medicine, Cardioangiology, and Hepatology, University Hospital S Orsola-Malpighi, Via Massarenti, 9, 40138 Bologna, Italy. E-mail: ravaglia@med.unibo.it.

Received April 28, 2006.

Accepted for publication August 3, 2006.

(MMSE; 23), and participants scoring <24 underwent further examination with the Mental Deterioration Battery (24). Dementia was diagnosed without further neuropsychological evaluation for participants with MMSE scores <10. Standardized information on the general functional and mental status of these subjects was also obtained from a collateral informant (a relative or other person with reliable knowledge of the subject, such as the subject's physician). Dementia was defined with the use of clinical criteria from the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (25). The same protocol was used to collect data on cognitive status at follow-up examination (in 2003–2004) for 676 persons who were free of dementia at baseline. For the other 124 persons who were free of dementia at baseline and who survived but declined to participate at follow-up or died before reexamination, information from multiple sources (the subjects themselves, relatives, general practitioners, and death certificates) was used to define the presence or absence of dementia.

Subjects were considered eligible for the present study only if they had a baseline MMSE score ≥ 24 and had not developed dementia by the time of follow-up, because all the main outcome measures are risk factors for this condition and the resulting confounding might obscure the association. Complete data were available for 671 of the 865 eligible persons (574 who undertook the follow-up examination and 97 who did not participate at follow-up but had adequate information for cognitive diagnosis). A flow chart that details the sample derivation is shown in **Figure 1**.

Written informed consent for the collection and use of this information was obtained from the subjects or their relatives. The study protocol was approved by the Isnstitutional Review Board of the Department of Internal Medicine, Cardioangiology, and Hepatology of the University of Bologna.

Laboratory investigations

At baseline, fasting venous blood samples were taken; they were immediately sent to the biochemical laboratory for processing. Serum folate and vitamin B-12 were measured with the use of immediate immunoelectrochemiluminescence analysis (Elecsys Folate Immunoassay and Elecsys B12 Immunoassay for Elecsys 2010 System; Roche Diagnostics Italia SpA, Monza, Italy). Serum total cholesterol and triacylglycerols were measured with the use of an enzymatic assay (Roche Diagnostics) on a Hitachi 917 System autoanalyzer (Boehringer Mannheim, Mannheim, Germany). Serum creatinine was measured by using the Jaffé method adapted for autoanalyzers. Serum CRP was measured with the use of the N–high-sensitivity CRP assay on a BN II analyzer (Dade Behring, Milan, Italy). Plasma tHcy was measured with the automated IMx assay (Abbott Laboratories, Abbott Park, IL) on plasma samples stored at −70 °C for ≤12 mo. Intraassay and interassay CVs for homocysteine, serum B vitamins, and serum CRP were reported in detail elsewhere (14, 26).

Apolipoprotein E genotypes

A commercial kit (QiAmp blood kit; Kaga, Crawley, United Kingdom) was used for genomic DNA extraction. Standardized polymerase chain reaction protocols were used for genotyping of *APOE* (27) and the 677 (C→T) polymorphism of the gene was used for methylenetetrahydrofolate reductase (MTHFR) (28), a key enzyme in homocysteine metabolism. The variant *T/T* genotype is associated with the highest homocysteine concentrations under conditions of impaired folate status (29). Subjects were categorized as carriers or noncarriers of *APOE4* and 677 *MTHFR T/T* genotypes.

Covariates

Covariates were selected on the basis of preliminary analyses (14, 28) and previous reports of homocysteine determinants (16, 17, 29) and were defined with the use of data collected at baseline. Educational status was categorized as 3 y compared with ≥4 y of formal education, because only a few participants had completed 5 y of formal education. Smoking habit was categorized as never smokers, ex-smokers, and current smokers. Physical activity was categorized as sedentary or active lifestyle, defined as performing at least moderate physical activity for a minimum of 4 h/wk. Diagnoses of cardiovascular disease (myocardial infarction, angina, peripheral vascular disease, congestive heart failure) and stroke were based on medical history provided by the patients and confirmed by their physicians. When available, previous medical records were also reviewed. Body mass index (in kg/m^2) was calculated.

Statistical analysis

Continuous variables are presented as means ± SDs, and categorical variables (except plasma tHcy, serum folate, serum vitamin B-12, and serum CRP because of their highly skewed distribution) are presented as number and percentage. Natural log–transformed values provided the best-fitting model for analyses in which plasma tHcy, serum folate, and serum vitamin B-12 were treated as continuous variables, and values were reported as geometric means and 95% CIs. Hyperhomocysteinemia was defined as plasma tHcy >15 μmol/L (14), which corresponds to the standard definition for hyperhomocysteinemia by international consensus (29). Because combined B vitamin deficits are more influential as a cause of hyperhomocysteinemia than are single deficits (16), we created a B vitamin index of 4 categories, based on the median value of each serum vitamin: combined B vitamin depletion (serum folate ≤5.3 ng/mL and serum vitamin B-12 ≤341 pg/mL), selective folate depletion (serum folate ≤5.3 ng/mL and serum vitamin B-12 >341 pg/mL), selective vitamin B-12 depletion (serum folate >5.3 ng/mL and serum vitamin B-12 ≤341 pg/mL), and B vitamin repletion (serum folate >5.3 ng/mL and serum vitamin B-12 >341 pg/mL). These thresholds fall near the upper limits of the range at which plasma tHcy is reported to begin increasing steeply [serum folate: 3–4.4 ng/mL (7–10 nmol/L); vitamin B-12: 271–339 pg/mL (200–250 pmol/L)] (29). As expected (16), the highest mean plasma concentration of tHcy (approximately corresponding to the cutoff for hyperhomocysteinemia) was found for combined B vitamin depletion (**Table 1**).

Serum CRP distribution was so skewed that none of the commonly used mathematical transformations, including logarithms, allowed a satisfactory normalization. Therefore, CRP was dichotomized at the median value (3 mg/L), which corresponds to a significant increase in cardiovascular disease risk (30), and analyzed only as a categorical variable.

We used *t* tests and chi-square tests for comparisons between groups. Factorial analysis [2 × 3 analysis of variance (ANOVA)] was used to assess the effect of *APOE4*, high CRP, and B vitamin

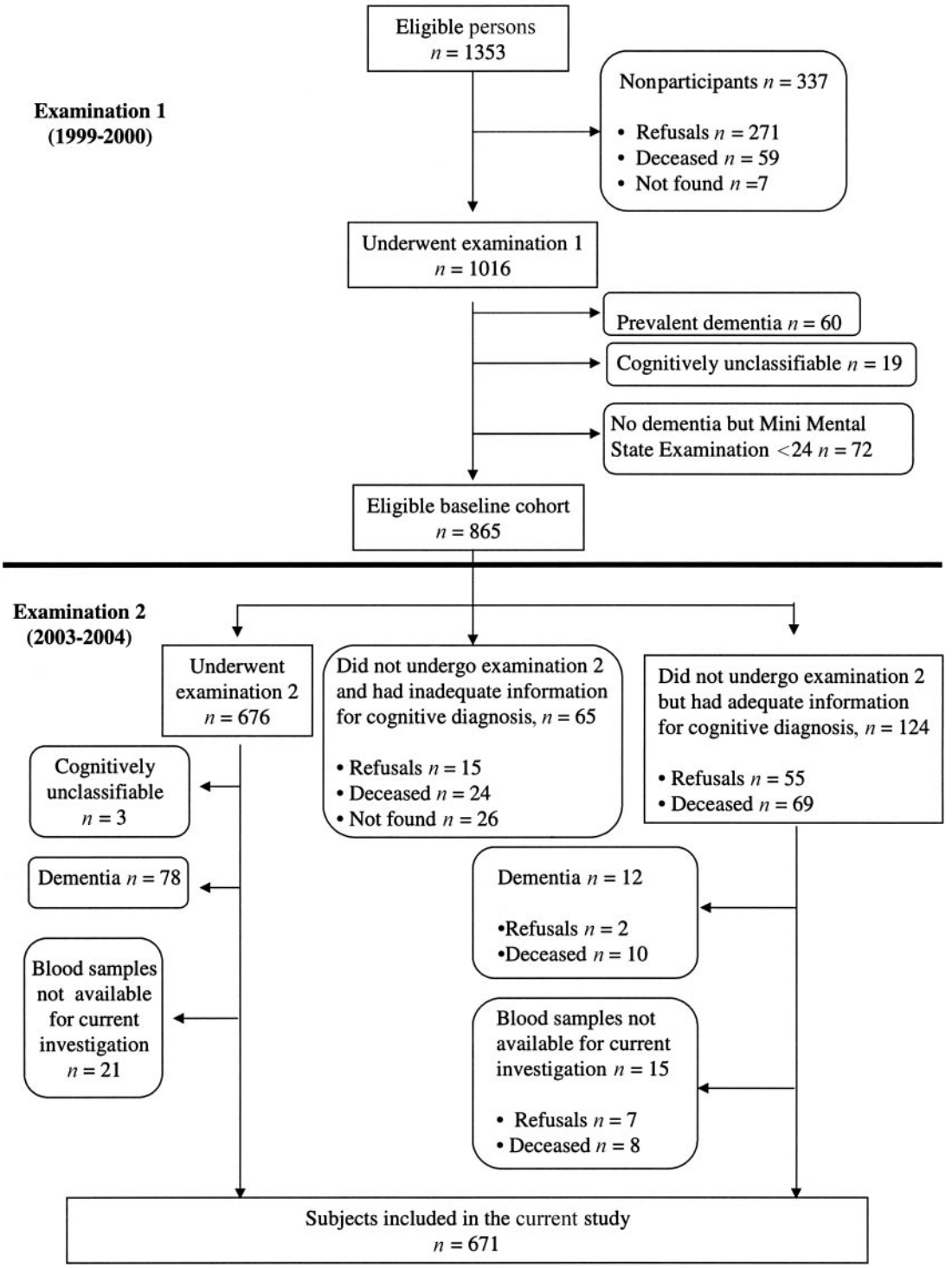

FIGURE 1. Flow chart detailing the derivation of the study sample.

status (categorical) on plasma tHcy. Because our primary concern was the concurrent effect of these variables on homocysteinemia, the most relevant statistics were the interaction terms *APOE4*× B vitamin status and *APOE4*× high CRP. In the event of a statistically significant interaction term, Tukey's test for all pairwise comparisons was used to compare the subgroups obtained, by stratifying the sample for both the variables included in the interaction term. The ANOVA model was also tested after adjustment for possible confounders. A first group of confounders (model 1) included all the study covariates giving a statistically significant contribution to the model (ie, age, sex, education, serum concentration of creatinine, physical activity, cardiovascular disease, stroke, and baseline MMSE). A second group of confounders (model 2) included all the variables of model 1 along with other variables that were biologically important variables without statistically significant effect on the model

TABLE 1
Mean plasma total homocysteine (tHcy) by categories of B vitamin status[1]

	Combined B vitamin depletion	Selective folate depletion	Selective vitamin B-12 depletion	B vitamin repletion	*P*
n	181	149	156	174	
Plasma tHcy (μmol/L)	14.8 (14.2, 15.4)[2,a]	12.8 (12.3, 13.4)[b]	12.0 (11.5, 12.5)[c]	11.1 (10.6, 11.5)[d]	< 0.001

[1] Values are estimated by using a one-factor ANOVA model adjusted for age, sex, and serum creatinine. Combined B vitamin depletion indicates serum folate ≤5.3 ng/mL and serum vitamin B-12 ≤341 pg/mL; selective folate depletion indicates serum folate ≤5.3 ng/mL and serum vitamin B-12 >341 pg/mL; selective vitamin B-12 depletion indicates serum folate >5.3 ng/mL and serum vitamin B-12 ≤341 pg/mL; and B vitamin repletion indicates serum folate >5.3 ng/mL and serum vitamin B-12 >341 pg/mL. Values with different superscript letters are significantly different, $P < 0.05$ (Tukey's test for all pairwise multiple comparisons).

[2] Geometric $\bar{x}$; 95% CIs in parentheses (all such values).

(ie, smoking habit, serum concentrations of total cholesterol and triacylglycerols, body mass index, and *MTHFR* genotype).

The effect of *APOE4* on CRP as a continuous variable could not be analyzed by using a factorial ANOVA design because of the failure to normalize its skewed distribution. However, the OR and 95% CI for the association of *APOE4* (dependent variable) with high CRP and hyperhomocysteinemia (independent variables) were estimated by using a logistic regression model that also included the interaction term high CRP × hyperhomocysteinemia. The interaction term was again the most relevant statistic of this analysis because our primary aim was to investigate whether and how each of the variables affected the association of *APOE4* with the other. In the event of the interaction term's being significant, ORs were estimated from the model according to the procedure described by Hosmer and Lemeshow (31). For this analysis, homocysteine was treated as a categorical variable to make it easier for the reader to understand the meaning of the ORs. Results were also tested by running additional logistic models adjusted for B vitamin status (categorical) and all of the other covariates used for multivariable adjustment of the ANOVA models. In preliminary analyses, the logistic model was also checked for interactions between B vitamin status (both as a categorical variable and as continuous log-transformed serum folate and vitamin B-12) and all the variables of interest. However, these interactions were not included in the final analysis because they were not significant ($P > 0.200$ for all) (31). Statistical analyses were performed with the use of SYSTAT software (version 10; SPSS Inc, Chicago, IL). Statistical significance was set at $P < 0.050$.

RESULTS

Plasma total homocysteine and *APOE* genotype

As expected, *e3/e3* was the most frequent *APOE* genotype ($n = 488$; 72.7%), and *e3/e4* ($n = 89$; 13.3%), *e3/e2* ($n = 77$; 11.5%), *e4/e2* ($n = 9$; 1.3%), *e2/e2* ($n = 3$; 0.4%), and *e4/e4* ($n = 5$; 0.7%) ranked next in descending order. The characteristics of study population by *APOE4* status are detailed in **Table 2**. Mean plasma tHcy and prevalence of hyperhomocysteinemia were lower in *APOE4* carriers than in noncarriers. No association with *APOE4* status was found for the other study variables. The lack of difference in plasma tHcy across the 677 *MTHFR* genotypes, either in the population as a whole or with respect only to those subjects with selective folate depletion ($P > 0.100$ for all), is in agreement with other results (28, 32), which suggests that the effect of the 677 *MTHFR* variant loses importance in older age.

The multivariable ANOVA model confirmed the significant effect of *APOE4* ($F = 6.073$, $P = 0.014$) and B vitamin status ($F = 18.455$, $P < 0.001$) on plasma tHcy and also showed that these variables are related to homocysteinemia independently of each other, because their interaction was not significant ($F = 1.470$, $P = 0.221$). In the same model, CRP was not significantly associated with homocysteinemia ($F = 0.402$, $P = 0.526$), but the interaction term *APOE4*× high CRP was significant ($F = 8.014$, $P = 0.005$), which suggests the need to investigate specific differences across plasma homocysteine concentrations stratified by *APOE4* and CRP. As shown in **Table 3**, the association between *APOE4* and lower plasma tHcy was confirmed only in subjects without concurrent high CRP.

Multivariate adjustment did not affect the statistical significance of *APOE4*× high CRP (model 1: $F = 8.014$, $P = 0.005$; model 2: $F = 8.821$, $P = 0.003$) or the results for the corresponding pairwise comparisons (*APOE4* carriers compared with noncarriers among subjects without high CRP: $P = 0.003$ for both models 1 and 2). *APOE4* noncarriers with low CRP had the highest homocysteine concentration but did not differ significantly from subjects with high CRP. Results did not change when log-transformed serum folate and vitamin B-12 were used instead of the categorical variable B vitamin status.

Information was also available about baseline alcohol and caffeine intakes, serum thyrotropin and plasma pyridoxal-5′-phosphate, diabetes, hypertension, and the use of nonsteroidal antiinflammatory drugs and statins. Adjustment for these additional variables did not alter the results (data not shown).

Association of *APOE4* with high serum C-reactive protein and effect of hyperhomocysteinemia

The unadjusted logistic model that examined the simultaneous association of high CRP and hyperhomocysteinemia with *APOE4* confirmed that the condition of being an *APOE4* carrier was associated with a lower risk of both high CRP (OR = 0.57; 95% CI: 0.36, 0.91) and hyperhomocysteinemia (OR = 0.25; 95% CI: 0.10, 0.65) than was the condition of being a noncarrier. However, the high CRP × hyperhomocysteinemia interaction term was also significant ($P = 0.034$) and remained so after multivariate adjustment ($P < 0.05$ for both models 1 and 2), which indicatied the need to estimate separate ORs for each

TABLE 2
Characteristics of the study population[1]

	APOE4 noncarriers (n = 568)	*APOE4* carriers (n = 103)	P[2]
Age (y)	72.9 ± 6.0[3]	72.0 ± 5.4	0.117
Female [n (%)][4]	291 (51.2)	56 (54.4)	0.558
Education ≤3 y [n (%)]	145 (25.5)	30 (29.1)	0.444
Plasma total homocysteine (μmol/L)	12.7 (12.4, 13.1)[5]	11.8 (11.0, 12.6)	0.035
Hyperhomocysteinemia [n (%)][6]	137 (24.1)	14 (13.6)	0.019
Serum folate (ng/mL)	5.2 (5.0, 5.4)	4.9 (4.4, 5.4)	0.241
Serum vitamin B-12 (pg/mL)	333 (318, 347)	318 (287, 352)	0.418
B vitamin status[7]			
Combined B vitamin depletion [n (%)]	145 (25.5)	36 (34.9)	0.178
Selective folate depletion [n (%)]	130 (22.9)	19 (18.4)	
Selective vitamin B-12 depletion [n (%)]	131 (23.1)	25 (24.3)	
B vitamin repletion [n (%)]	162 (28.5)	23 (22.3)	
677 *MTHFR T/T* genotype [n (%)]	119 (20.9)	20 (19.4)	0.825
Serum C-reactive protein > 3 mg/L [n (%)]	291 (51.2)	44 (42.7)	0.112
Serum creatinine (mg/dL)	0.99 ± 0.22	0.96 ± 0.20	0.126
Serum total cholesterol (mg/dL)	240 ± 39	246 ± 41	0.180
Serum triacylglycerols (mg/dL)	122 ± 61	124 ± 64	0.788
Smoking status			
Smokers [n (%)]	56 (9.9)	10 (9.7)	0.860
Ex-smokers [n (%)]	191 (33.6)	32 (31.1)	
Never-smokers [n (%)]	321 (56.5)	61 (59.2)	
Active lifestyle [n (%)]	356 (62.7)	66 (64.1)	0.786
BMI (kg/m^2)	28.9 ± 4.4	28.3 ± 4.0	0.223
Cardiovascular disease [n (%)]	100 (17.6)	16 (15.5)	0.609
History of stroke [n (%)]	15 (2.6)	2 (1.9)	0.678
Baseline Mini Mental State Examination (score)	28.3 ± 1.4	28.5 ± 1.2	0.174

[1] *APOE4, apolipoprotein E e4* allele; tHcy, total homocysteine; *MTHFR,* methylenetetrahydrofolate reductase.
[2] P values for differences between the groups were calculated by using t test (continuous variables) and chi-square test (categorical variables).
[3] $\bar{x}$ ± SD (all such values).
[4] Percentage values are categorical variables.
[5] Geometric $\bar{x}$; 95% CIs in parentheses (all such values).
[6] Hyperhomocysteinemia indicates total plasma homocysteine >15 μmol/L.
[7] Combined B vitamin depletion indicates serum folate ≤5.3 ng/mL and serum vitamin B-12 ≤341 pg/mL; selective folate depletion indicates serum folate ≤5.3 ng/mL and serum vitamin B-12 >341 pg/mL; selective vitamin B-12 depletion indicates serum folate >5.3 ng/mL and serum vitamin B-12 ≤341 pg/mL; B vitamin repletion indicates serum folate >5.3 ng/mL and serum vitamin B-12 >341 pg/mL.

variable stratified by the other. As shown in **Table 4**, the probability of high CRP was lower for *APOE4* carriers than for noncarriers only if the carriers were without hyperhomocysteinemia. Conversely, in agreement with the results from the ANOVA model, the probability of hyperhomocysteinemia was lower for *APOE4* carriers than for noncarriers but only if the carriers were without high CRP. Among subjects with both high CRP and hyperhomocysteinemia, the prevalence of high CRP tended to be higher in *APOE4* carriers than in noncarriers, but the association was not significant.

TABLE 3
Mean plasma total homocysteine (tHcy) by *APOE4* carrier status and serum C-reactive protein (CRP)[1]

	Low CRP[2]		High CRP[3]		
	APOE4 noncarriers	*APOE4* carriers	*APOE4* noncarriers	*APOE4* carriers	P for *APOE4* × CRP interaction
n	277	59	291	44	
Plasma tHcy (μmol/L)	13.6 (13.1, 14.2)[4,a]	12.1 (11.6, 12.5)[b]	12.3 (11.4, 13.3)[a,b]	12.2 (11.1, 13.5)[a,b]	0.005

[1] Values are estimated from a multifactorial design (2 × 3 ANOVA), including *APOE4,* CRP, and B vitamin status (categorical). Values with different superscript letters are significantly different, $P < 0.001$ [post hoc analysis of the significant interaction term *APOE4* × CRP (Tukey's test for all pairwise multiple comparisons)].
[2] Defined as CRP ≤3 mg/L.
[3] Defined as CRP >3 mg/L.
[4] Geometric $\bar{x}$; 95% CIs in parentheses (all such values).

TABLE 4
Relation of *APOE4* carrier status with high C-reactive protein (CRP; >3 mg/L) and hyperhomocysteinemia (plasma total homocysteine >15 μmol/L)[1]

	Cases[2]	Unadjusted OR (95% CI)	Model 1[3] OR (95% CI)	Model 2[4] OR (95% CI)
	n (%)			
High CRP				
Without hyperhomocysteinemia				
APOE4 carriers (*n* = 89)	54 (39.1)	0.57 (0.36, 0.91)	0.50 (0.26, 0.34)	0.51 (0.31, 0.85)
APOE4 noncarriers (*n* = 431)	229 (53.1)	Reference	Reference	Reference
With hyperhomocysteinemia				
APOE4 carriers (*n* = 14)	9 (64.3)	2.18 (0.69, 6.89)	2.05 (0.64, 6.60)	2.21 (0.68, 7.19)
APOE4 noncarriers (*n* = 137)	62 (45.3)	Reference	Reference	Reference
Hyperhomocysteinemia				
Without high CRP				
APOE4 carriers (*n* = 59)	5 (8.5)	0.25 (0.10, 0.64)	0.23 (0.08, 0.61)	0.22 (0.08, 0.59)
APOE4 noncarriers (*n* = 277)	75 (27.1)	Reference	Reference	Reference
With high CRP				
APOE4 carriers (*n* = 44)	9 (20.4)	0.65 (0.30, 1.42)	0.62 (0.25, 1.55)	0.61 (0.24, 1.53)
APOE4 noncarriers (*n* = 291)	61 (20.4)	Reference	Reference	Reference

[1] OR, odds ratio; *APOE4, apolipoprotein E e4* allele. Unadjusted ORs were estimated from a logistic model by using *APOE4* as the dependent variable and including hyperhomocysteinemia, CRP, and an interaction term high CRP × hyperhomocysteinemia as the main independent variables. The interaction term high CRP × hyperhomocysteinemia was significant for each model ($P < 0.05$), which indicated the need to stratify each variable for the other.

[2] Refers to subjects with high CRP or with hyperhomocysteinemia.

[3] Adjusted as model 1 and additionally adjusted for age, sex, education, serum creatinine, B vitamin status (categorical), active lifestyle, cardiovascular disease, history of stroke, serum CRP, and baseline Mini Mental State Examination.

[4] Additionally adjusted for all the covariates of model 1 plus smoking, serum cholesterol, serum triacylglycerols, BMI, and *677 methylenetetrahydrofolate reductase T/T* genotype carrier status.

DISCUSSION

The current study showed that, in an elderly population, hyperhomocysteinemia was less prevalent among *APOE4* carriers than among noncarriers but only if the carriers were without high CRP. Moreover, the study confirmed and extended previous sporadic reports (4–7) of an association between *APOE4* and lower prevalence of high CRP, which showed that the association was limited to subjects without hyperhomocysteinemia. All associations were found to be independent of B vitamin status.

A possible explanation for our findings is that, as has been hypothesized for VLDL-bound CRP (5), *APOE4* affects the clearance of the lipoprotein-bound homocysteine fraction. Approximately 80% of homocysteine circulates in blood bound to proteins, with albumin as the main carrier (29). However, ≈27% of all protein-bound homocysteine circulates bound to lipoproteins, and VLDL has the highest binding capacity, possibly because of its apolipoprotein B content (18–20). *APOE4* carriers have the highest apolipoprotein B concentrations and are particularly efficient in clearing VLDL from blood (1, 2). Therefore, under conditions leading to hyperhomocysteinemia, *APOE4* carriers may have greater binding of homocysteine to lipoproteins, a faster clearance of the lipoprotein-bound homocysteine fraction, or both. Further support to the hypothesis is the fact that the association of *APOE4* with a lower risk of hyperhomocysteinemia weakens under conditions of high CRP, whereas the association of *APOE4* with a lower risk of high CRP weakens under conditions of hyperhomocysteinemia. These facts combine to suggest that CRP and homocysteine may compete for the same mechanism (perhaps a lipoprotein carrier) that favors their blood clearance in *APOE4* carriers. Alternatively, *APOE4* may be in linkage disequilibrium with other unknown genes that are able to affect both homocysteine and CRP metabolism.

The results that *1*) the highest homocysteine concentration was actually found in *APOE4* noncarriers with low CRP and *2*) *APOE4* carriers with high CRP had average homocysteine concentrations not significantly different from those in both *APOE4* carriers and noncarriers with low high CRP may seem inconsistent with the hypothesis that high CRP attenuates the homocysteine-lowering effect of *APOE4*. However, by looking at the percentage of subjects with hyperhomocysteinemia in each group, it can be observed that the figures for subjects with high CRP and for *APOE4* noncarriers with low CRP are almost 3 times the figures for *APOE4* carriers with low CRP. Therefore, it may be speculated that, above a certain threshold of homocysteinemia, the hypothesized *APOE4*-related mechanism for removal of the homocysteine-lipid bound fraction is saturated, and other, more efficient, catabolic pathways are activated. That possibility may explain why the subjects with high CRP had lower homocysteinemia than expected: that is, the concurrent presence of high CRP so attenuates the efficiency of lipid-bound homocysteine removal that, independently of *APOE*, additional catabolic pathways are triggered. At the same time, that possibility may justify the unexpectedly high homocysteinemia found in *APOE4* noncarriers with low CRP: that is, they lack the *APOE4*-related advantage for removal of the homocysteine lipid-bound quote, but their low CRP does not interfere with the normal VLDL or VLDL-like mechanism, so the threshold for activation of the additional mechanisms is not reached. Of course, because of the scant numbers in the *APOE4* carrier subgroups, it cannot be excluded that the post hoc all-pairwise comparison test failed to identify existent significant differences.

No association between homocysteinemia and *APOE4* was reported in previous investigations of Polish (33) or adult Taiwanese (34) subjects. Those investigations, however, were

not specifically focused on the association of interest, and they had small population sizes. Differences in *APOE4* frequencies related to differences in the ethnic origin of the samples (1) and to the selection criteria used for the present investigation—that is, *APOE4* and other genotypes associated with risk of cognitive impairment are likely to be underrepresented in this study sample because of the exclusion of subject with prevalent and incident dementia—and to the better B vitamin status of the Polish population may have contributed to the different results obtained in those studies.

The main strengths of the current investigation are its population size, the large number of covariates available in the CSBA database, and the specific selection of persons free of dementia both at baseline and after a 4-y follow-up, which avoids the confounding effect of known associations between cognitive status and the variables of interest. The study, however, has several limitations. First, results for the analysis examining the interactions of hyperhomocysteinemia and CRP in their association with *APOE4* are based on relatively small numbers of subjects. Second, because of its skewed distribution, CRP could be analyzed only as a categorical variable. Third, serum folate and vitamin B-12 are not the best indicators of metabolic vitamin deficiency, and other unmeasured vitamins may influence homocysteine metabolism (35). Fourth, only a single measurement of laboratory values was available. Fifth, although *APOE4*, homocysteine, and CRP are all associated with a greater risk of atherosclerotic events, we did not systematically excluded persons with vascular disease. The CSBA design did not include an assessment of cardiovascular conditions beyond clinical diagnosis, and it relied on data provided by participants and their physicians. Moreover, incident atherothrombotic events were not actively sought among those persons who refused to participate or who died before follow-up. However, the presence of vascular conditions at baseline was considered among the covariates, and inclusion of incident vascular events in the models as an additional covariate did not substantially change the results (data not shown). Sixth, although we did not genotype the cohort for known CRP genetic polymorphisms, evidence suggests that these polymorphisms have no effect on the association between *APOE4* and serum CRP (6). Seventh, it may be speculated that the somewhat paradoxical association between a genetic risk factor for dementia and the lower concentrations of putative dementia risk factors that we found in this sample is the reason that the *APOE4* carriers included in the study did not develop dementia during follow-up. In particular, it would have been interesting to verify whether the association was also present in the CSBA subjects who were cognitively normal at baseline but who received a diagnosis of dementia at follow-up and were excluded from the present investigation. However, the smallness of the latter group (only 12 subjects with full laboratory data) did not allow any reliable statistical analysis, and further studies on larger samples and with longer follow-up time are needed to investigate the hypothesis that blood concentrations of homocysteine and CRP may affect the risk of Alzheimer dementia associated with *APOE4*. A larger study sample is also needed to look for a confirmation of the nonsignificant trend we observed, under condition of hyperhomocysteinemia, for *APOE4* carriers to have a higher prevalence of high CRP than noncarriers, because these subjects may be at greater risk of dementia.

In conclusion, this study suggests that, in comparison with noncarriers, *APOE4* carriers have, independently from B vitamin status, a lower risk of hyperhomocysteinemia and of high CRP. However, the presence of one condition attenuates the association of *APOE4* with the other, and it cannot be excluded that, for substantial increases in CRP and homocysteine, their hypothesized competition at a common *APOE4*-related mechanism may be bypassed by the intervention of other, more efficient elimination pathways. Given the growing interest of researchers in homocysteine and CRP as risk factors for vascular events and dementia (2, 3, 8–10), our results suggest the need to take the *APOE* genotype into account in future studies that involve blood measurement of these markers.

We thank Edoardo Dalmonte (head physician of the Division of Geriatric Medicine at the Ravenna Hospital) and the general practitioners of Conselice for their invaluable help in organizing the study. We also thank the population of Conselice for their enthusiasm and participation in the study.

GR, PF, and FMa were responsible for the conception and design of the study; GR was responsible for obtaining the funds for the study; GR is responsible for the integrity of the data and the accuracy of the data analysis; PF, MC, and FMa were responsible for data collection; PF and FMo were responsible for data analysis, with the assistance of MC, FMa, and CP; GR, PF, and FMa were responsible for drafting the manuscript; and FL, MB, and CP contributed to the revision of the manuscript. None of the authors had a financial or personal conflict of interest.

REFERENCES

1. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000;1:507–37.
2. Eichner JE, Dunn ST, Perveen GP, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002;155:487–95.
3. Poirer J. Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. Neurobiol Aging 2005;26:355–61.
4. Manttari M, Manninen V, Palosuo T, Ehnholm C. Apolipoprotein E polymorphism and C-reactive protein in dyslipidemic middle-aged men. Atherosclerosis 2001;156:237–8 (letter).
5. Austin MA, Zhang C, Humphries SE, et al. Heritability of C-reactive protein and association with apolipoprotein E genotypes in Japanese Americans. Ann Intern Med 2004;68:179–88.
6. Judson R, Brain C, Dain B, Windemuth A, Ruano G, Reed C. New and confirmatory evidence of an association between APOE genotype and baseline C-reactive protein in dyslipidemic individuals. Atherosclerosis 2004;177:345–51.
7. Marz W, Scharnagl H, Hoffmann MM, Boehm BO, Winkelmann BR. The apolipoprotein E polymorphism is associated with circulating C-reactive protein (the Ludwigshafen risk and cardiovascular health study). Eur Heart J 2004;25:2109–19.
8. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. Am J Med 2004;116(suppl): 9S–16S.
9. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. Ann Neurol 2002;52:168–74.
10. Engelhart MJ, Geerling MI, Meijer J, et al. Inflammatory proteins in plasma and the risk of dementia. The Rotterdam Study. Arch Neurol 2004;61:668–72.
11. Schwalbe RA, Coe JE, Nelsestuen GL. Association of rat C-reactive protein and other pentraxins with rat lipoproteins containing apolipoproteins E and A1. Biochemistry 1995;34:10432–9.
12. Rowe IF, Soutar AK, Trayner IM, Thompson GR, Pepys MB. Circulating human C-reactive protein binds very low density lipoproteins. Clin Exp Immunol 1984;58:237–44.
13. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
14. Ravaglia G, Forti P, Maioli F, et al. Homocysteine and folate as risk factors for dementia and Alzheimer Disease. Am J Clin Nutr 2005;82: 636–43.
15. The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke. A meta-analysis. JAMA 2002;288: 2015–22.

16. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
17. Herrmann W, Quast S, Ullrich M, Schultze H, Bodis M, Geisel J. Hyperhomocysteinemia in high-aged subjects: relation of B-vitamins, folic acid, renal function and the methylenetetrahydrofolate reductase mutation. Atherosclerosis 1999;144:91–100.
18. McCully KS, Olszewski AJ, Vezeridis MP. Homocysteine and lipid metabolism in atherogenesis: effect of the homocysteine thiolactonyl derivatives, thioretinaco and thioretinamide. Atherosclerosis 1990;83: 197–206.
19. Olszewski AJ, McCully KS. Homocysteine content of lipoproteins in hypercholesterolemia. Atherosclerosis 1991;88:61–8.
20. Ventura P, Panini R, Rosa MC, Gaetti E, Salvioli G. Relevance of different apolipoprotein content in binding of homocysteine to plasma lipoproteins. Nutr Metab Cardiovasc Dis 2003;4:218–26.
21. Ravaglia G, Forti P, Maioli F, et al. Education, occupation, and prevalence of dementia: findings from the Conselice Study. Dement Geriatr Cogn Disord 2002;14:90–100.
22. Ravaglia G, Forti P, Maioli F, et al. Conselice study: a population based survey of brain aging in a municipality of the Emilia Romagna region: (A.U.S.L. Ravenna). Design and methods. Arch Gerontol Geriatr 2001; 7:313–24.
23. Valente C, Maione P, Lippi A, et al. (Validation of the Mini-Mental State Examination (MMSE) as a screening instrument for dementia in an Italian population.) G Gerontol 1992;40:161–5 (in Italian).
24. Carlesimo GA, Caltagirone C, Gainotti G. The Mental Deterioration Battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment. The Group for the Standardization of the Mental Deterioration Battery. Eur Neurol 1996;36:378–84.
25. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association, 1994.
26. Ravaglia G, Forti P, Maioli F, et al. Plasma homocysteine and inflammation in elderly patients with cardiovascular disease and dementia. Exp Gerontol 2004;39:443–50.
27. Licastro F, Pedrini S, Ferri C, et al. Gene polymorphism affecting α1-antichymotrypsin and interleukin-1 plasma levels increases Alzheimer's disease risk. Ann Neurol 2000;48:388–91.
28. Ravaglia G, Forti P, Maioli F, et al. Common polymorphisms in methylenetetrahydrofolate reductase (MTHFR): relationships with plasma homocysteine concentrations and cognitive status in elderly Northern Italian subjects. Arch Gerontol Geriatr Suppl 2004;9:339–48.
29. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3–32.
30. Ridker PM, Wilson PWF, Grundy SM. Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? Circulation 2004;109:2818–25.
31. Hosmer DW, Lemeshow S. Applied logistic regression. New York, NY: Wiley, 1989.
32. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996;94:2410–6.
33. Religa D, Styczynska M, Peplonska B, et al. Homocysteine, apolipoproteine E and methylenetetrahydrofolate reductase in Alzheimer's disease and mild cognitive impairment. Dement Geriatr Cogn Disord 2003;16: 64–70.
34. Lin SK, Kao JT, Tsai SM, et al. Association of apolipoprotein E genotypes with serum lipid profiles in a healthy population of Taiwan. Ann Clin Lab Sci 2004;34:443–8.
35. Jacques PK, Kalmbach R, Bagley PJ, et al. The relation between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677 transition in the methylenetetrahydrofolate reductase gene. J Nutr 2002;132:283–8.