Apolipoprotein E-related all-cause mortality in hospitalized elderly patients

Maria G. Matera · Daniele Sancarlo · Francesco Panza · Carolina Gravina ·
Grazia D'Onofrio · Vincenza Frisardi · Grazia Longo · Luigi P. D'Ambrosio ·
Filomena Addante · Massimiliano Copetti · Vincenzo Solfrizzi · Davide Seripa ·
Alberto Pilotto

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Abstract The most common apolipoprotein E (APOE) allelic variation is implicated in many age-related diseases and human longevity with controversial findings. We investigated the effect of APOE gene polymorphism on all-cause mortality in elderly patients taking into consideration the functional disability, cognitive impairment, malnutrition, and the occurrence of common age-related diseases. APOE genotypes were determined in 2,124 geriatric hospitalized patients (46.5% men and 53.5% women; mean age, 78.2 ± 7.1 years; range, 65–100 years). At hospital admission, all patients underwent a comprehensive geriatric assessment to evaluate functional disability, cognitive status,

nutritional status, and comorbidity. The main and secondary diagnoses at hospital discharge were also recorded. Mortality status was evaluated in all patients after a maximum follow-up of 5 years (range, from 1.26 to 5.23 years; median, 2.86 years). During the study period, 671 patients died (32.0%). At hospital admission, these patients showed a significant higher prevalence of cardiovascular diseases (56.3% vs 53.4%; $p=0.007$), neoplasias (32.3% vs 13.7%; $p<0.001$), and lower prevalence of neurodegenerative diseases (17.7% vs 20.7%; $p<0.001$) than survived patients. Moreover, they also showed an higher prevalence of disability (52.0% vs 25.6%; $p<0.001$), cognitive impairment (31.0% vs 18.8%; $p<0.001$), and malnutrition (74.0% vs 46.1%; $p<0.001$) than survived patients. In the overall study population, the APOE $\epsilon 2$ allele was significantly associated to neurodegenerative diseases (odds ratio=0.59; 95% confidence interval (CI), 0.37–0.94). No significant association between the APOE polymorphism and disability, malnutrition, comorbidity status, and with all-cause mortality was observed. In patients with cardiovascular diseases, however, a decreased risk of all-cause mortality was found in the $\epsilon 2$ allele carriers (hazard ratio=0.56; 95% CI, 0.36–0.88). In this population, APOE allele variants might play a role on cardiovascular disease-related mortality.

M. G. Matera · D. Sancarlo · F. Panza · C. Gravina ·
G. D'Onofrio · G. Longo · L. P. D'Ambrosio · F. Addante ·
D. Seripa · A. Pilotto (✉)
Department of Medical Sciences,
Geriatrics Unit & Gerontology-Geriatric Research
Laboratory, IRCCS "Casa Sollievo della Sofferenza",
San Giovanni Rotondo,
Foggia, Italy
e-mail: alberto.pilotto@operapadrepio.it

V. Frisardi · V. Solfrizzi
Department of Geriatrics, Center for Aging Brain,
Memory Unit, University of Bari,
Bari, Italy

M. Copetti
Unit of Biostatistics,
IRCCS "Casa Sollievo della Sofferenza",
San Giovanni Rotondo,
Foggia, Italy

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Introduction

Previous studies on heritability of human longevity showed that genetic factors may account, approximately, for 20–30% of the overall variation in adult lifespan (Herskind et al. 1996; Iachine et al. 1998; De Benedictis et al. 2001; Hjelmborg et al. 2006). The genetic influence on lifespan appears to be greater at extreme aging (Perls et al. 1998), and individuals living to extreme old age are healthy and independent for most of their lives (Hitt et al. 1999). Although longevity might show Mendelian inheritance, aging and the susceptibility to age-related diseases may be influenced by environment, lifestyle, and probably by thousands of genes (Perls et al. 2002).

The exact identity of aging and longevity genes is unknown. In the quest to understand the biologic basis of human longevity, it can be supposed that genes and biochemical markers likely implied in coronary artery disease, cerebrovascular disease, and Alzheimer's disease (AD) may have a role in human longevity (Panza et al. 2007). The apolipoprotein E (APOE) gene polymorphism is one of the most studied gene variation in the world; its most common allelic variations of APOE gene ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) have been associated with many common age-related diseases. In fact, the APOE gene has been widely examined in different studies, because of its well-documented role in dementia as well as vascular diseases. It has been identified as a genetic risk factor for cognitive decline and AD (Hsiung et al. 2004; Mahley et al. 2006) and cardiovascular diseases (Song et al. 2004; Licastro et al. 2004), and it has been shown as a risk modulator of many other disorders (Jofre-Monseny et al. 2008). Notably, a relevant number of reports on human longevity showed APOE $\epsilon 4$ allele frequency being lower in older age groups (centenarians, octogenarians, and nonagenarians) than younger or middle-aged subjects (Lewis and Brunner 2004). The $\epsilon 2$ allele conversely has a higher frequency in younger than older population (Panza et al. 2007), and it has been suggested to decrease the risk for major age-related diseases, including AD, while its role in the cardiovascular diseases is not clear (Song et al. 2004).

At present, the effect of APOE polymorphism on mortality in the general population is not clear, and several studies reported conflicting results (Corder et al. 1996; Koivisto et al. 2000; Frisoni et al. 2001;

Slooter et al. 2001; Hayden et al. 2005). Moreover, differences in the association between this polymorphism and mortality have been reported across European (Ewbank 2004) and other populations (Ewbank 2007). Aim of the present study was to explore the potential association of APOE alleles on all-cause mortality in a large population of Caucasian hospitalized elderly patients from Central and Southern Italy, taking also into account the functional disability, cognitive impairment, malnutrition, and the occurrence of common age-related diseases.

Methods

Study population The study was conducted according to the Declaration of Helsinki and the guidelines for Good Clinical Practice and was approved by the local Ethics Committees. Patients were recruited from the Geriatric Unit of our Institution, in a frame of a project investigating the incidence of genetic risk factors for multidimensional impairment of elderly in Italy. All subjects were Caucasian and originating from Central and Southern Italy. Inclusion criteria were (1) age ≥ 65 years, (2) written informed consent obtained from the patient or from relatives of critically ill patients, and (3) availability of genetic data.

From January 2004 to December 2007, 2,792 patients, consecutively admitted to the Geriatric Unit of the IRCCS “Casa Sollievo della Sofferenza,” San Giovanni Rotondo, Italy, were screened for eligibility. From this population, 233 patients were excluded because they were younger than 65 years old, 52 patients did not give their informed consent, and 383 patients were excluded because information about their mortality/survival status was not available. Vital status up to March 31, 2009 was assessed by directly contacting the participants or consulting the Registry Offices of the cities where the patients were residents at the time of hospital admission. Dates of death were identified from death certificates.

Comprehensive geriatric assessment and clinical diagnoses At baseline, the following parameters were collected by structured interview, clinical evaluation, and review of records from the patients' general practitioners: date of birth, gender, clinical history, current pathologies, and medication history. Data on

demographic variables and medical history were obtained from patients or their caregivers at the baseline interview.

A comprehensive geriatric assessment (National Institutes of Health Consensus Development Conference Statement 2003) was carried out to determine the patient's functional and cognitive capacity, comorbidity, nutritional status, drug use, and social problems. Functional status was evaluated using the activities of daily living (ADL) index (Katz et al. 1970), which defines the level of dependence/independence of six daily personal care activities, including bathing, toileting, feeding, dressing, urine and bowel continence, and transferring in and out of bed or chair (score 0–6). Cognitive status was evaluated using the short portable mental status questionnaire (SPMSQ) (Pfeiffer 1975), a ten-item questionnaire evaluating orientation, memory, attention, calculation, and language (score 0–10). The nutritional status was evaluated by means of the mini nutritional assessment (MNA; Guigoz and Vellas 1999), which includes information regarding anthropometric measures (body mass index, body weight/height², mid-arm circumference in centimeters, calf circumference in centimeters, and weight loss), lifestyle, medication, and mobility, number of meals, food, and fluid intake, and autonomy of feeding and self-perception of health and nutrition. A 30-point scoring system was proposed for the MNA, with an MNA score ≥ 24 points identifying a normal nutrition status, an MNA score of 17–23.5 points that suggested a borderline status/at risk for malnutrition, and an MNA score ≤ 17 points identifying malnutrition (Guigoz and Vellas 1999). Comorbidity was evaluated by the cumulative illness rating scale (CIRS) (Linn et al. 1968). It uses five-point ordinal scales (score 1–5) to estimate the severity of pathology in each of 13 systems, including cardiac, vascular, respiratory, eye-ear-nose-throat, upper and lower gastroenteric diseases, hepatic, renal, genitourinal, musculoskeletal, skin disorders, nervous system, and endocrine metabolic and psychiatric behavioral problems. Based on the ratings, the comorbidity index (CI) reflects the number of concomitant diseases, and it is derived from the total number of categories in which moderate or severe levels (grades from 3 to 5) of disease are quoted (range from 0 to 13). A CIRS-CI score of 0 indicated no concomitant diseases, a CIRS-CI score from 1 to 2 indicated one or two concomitant diseases, and a

CIRS-CI score ≥ 3 indicated more than three concomitant diseases. The main and secondary diagnoses at discharge from the hospital, coded according to the Italian translation of the International Classification of Diseases, ninth revision, Clinical Modification (ICD-9-CM; available at URL: <http://icd9cm.chrisendres.com/icd9cm/>), were also recorded in all patients. In particular, cardiovascular diseases included the following diseases classified as ICD-9-CM code: 401–405, 410–414, and 415–429; neoplasia included diseases classified as ICD-9-CM code 140–239; and neurodegenerative diseases included diseases classified as ICD-9-CM code: 330–337.

Genotype analysis Genomic DNA was manually purified from peripheral blood by organic protein extraction and ethanol precipitation according to standard method. The APOE genotypes were determined by polymerase chain reaction (PCR) and agarose gel electrophoresis as recently described (Seripa et al. 2006a). Briefly, a combination of four specific primers in three different pairs sharing the same stringent PCR conditions was used. Allele-specific primers were ASP1 (CGG ACA TGG AGG ACG TGT), ASP2 (CGG ACA TGG AGG ACG TGC), ASP3 (CTG GTA CAC TGC CAG GCG), and ASP4 (CTG GTA CAC TGC CAG GCA), synthesized by Invitrogen (Invitrogen Corporation, Carlsbad, CA, USA). The primer pair ASP-1/ASP-4 identifies the $\epsilon 2$ allele, the primer pair ASP-1/ASP-3 identifies the $\epsilon 3$ allele, and the primer pair ASP-2/ASP-3 identifies the $\epsilon 4$ allele. On an Applied Biosystems GeneAmp PCR System 9700, the amplification conditions were 94°C for 2 min, followed by 32 cycles at 96°C for 15 s, 61°C for 30 s, and 72°C for 30 s. Reaction buffer included 1.5 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, Carlsbad, CA, USA), 10 pmol of each primer, 100 μ M NTPs, and 1 mM MgCl₂. The presence/absence of specific allele PCR products detected by electrophoresis analysis on a 2% agarose gel identified the six APOE genotypes.

Statistical analysis Normal distribution of continuous variables was checked using the Kolmogorov–Smirnov test. Comparisons across groups were evaluated using unpaired *t* test or one-way analysis of variance model for continuous variables and Pearson's chi-square test for categorical variables.

In the analysis of the APOE polymorphism, we considered allele carriers according to the three common human APOE: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles. The $\epsilon 3/\epsilon 3$ genotype was the reference category. $\epsilon 2$ - and $\epsilon 4$ carriers were considered subjects with those genotypes showing at least one APOE allele, i.e., subjects with the $\epsilon 2/\epsilon 2$ or $\epsilon 2/\epsilon 3$ genotypes ($\epsilon 2$ carriers) and subjects with $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$ genotypes ($\epsilon 4$ carriers). Carriers of $\epsilon 2/\epsilon 4$ genotypes were excluded from the analysis.

Variables were dichotomized as follows: subjects with an ADL score=6 were classified as subjects with no functional disability, and subjects with ADL score ≤ 6 were classified as subjects with functional disability; subjects with SPMSQ score ≥ 3 were classified as subject with cognitive impairment, and those with the score ≤ 3 were classified as subject without cognitive impairment; subjects with an MNA score ≥ 24 had a satisfactory nutritional status, while score ≤ 24 identified subject with non-satisfactory nutritional status (at risk for malnutrition status and malnutrition status were pooled); and subjects with CIRS=0 were classified as having no co-morbidity with respect to subjects with CIRS ≥ 0 . Associations between diseases and impairment in physical, cognitive, and nutritional status across APOE carriers at baseline were estimated using univariate logistic regression models. Results were reported as odds ratios (OR) along their 95% confidence interval (CI). Multivariate logistic models were built using a stepwise variable selection with p value for entry and stay in the model ≤ 0.20 and adjusting for age and gender.

Survival time was calculated in years from entry into the study until the time of death or the end of follow-up. Differences in survival probabilities were analyzed by comparing Kaplan–Meier survival curves using the log rank test. Furthermore, a multivariate Cox model was estimated using a stepwise variable selection, as above described, to identify which demographic or clinical variables contribute significantly to the model fit. Risks were reported as hazard ratios (HR) along their 95% CI. In all the analyses, the level of statistical significance was set at a p value ≤ 0.05 . Agreement of the observed genotype frequencies with the expected Hardy–Weinberg frequencies were verified with Arlequin Software (Schneider et al. 2000; Kocsis et al. 2004). All other statistical analyses were made

with the SPSS Ver. 10.1.3 statistical software package (SPSS Inc., Chicago, IL, USA).

Results

The complete data of APOE genotyping were available for 2,124 subjects selected for the study. The overall frequencies of the APOE genotypes were 10.4% for the $\epsilon 2/\epsilon 3$ genotype ($n=220$), 1.2% for the $\epsilon 2/\epsilon 4$ genotype ($n=25$), 72.1% for the $\epsilon 3/\epsilon 3$ genotype ($n=1,531$), 15.6% for the $\epsilon 3/\epsilon 4$ genotype ($n=331$), 0.7% for the $\epsilon 4/\epsilon 4$ genotype ($n=15$), and 0.1% for the $\epsilon 2/\epsilon 2$ genotype ($n=2$). These frequencies were not different from the expected Hardy–Weinberg frequencies ($p=0.16$). Estimated allele frequencies were 0.059 for the $\epsilon 2$ allele, 0.850 for the $\epsilon 3$ allele, and 0.091 for the $\epsilon 4$ allele.

After APOE genotyping, 25 patients with $\epsilon 2/\epsilon 4$ genotype were excluded from the analyses; thus, a total of 2,099 unrelated subjects (977 men and 1,122 women; mean age, 78.21 ± 7.11 years; range, from 65 to 100 years) were selected for the study. At baseline, women were significantly older (78.7 ± 7.2 vs 77.7 ± 7.0 years, $p=0.002$) and showed a major impairment in physical disability (ADL, 36.8% vs 30.8%; $p=s0.004$), cognitive functions (SPMSQ, 26.2% vs 18.5%; $p \leq 0.001$), and nutritional status (MNA, 60.6% vs 48.1%; $p \leq 0.001$) than men. Conversely, men showed a significant higher frequency of neoplasia than women (25.1% vs 15.0%; $p \leq 0.001$).

Dividing patients by their APOE genotype (Table 1), no significant differences in mean age, gender, and in the prevalence of cardiovascular diseases, neoplasias, physical disability, comorbidity, and malnutrition status were observed in $\epsilon 2$ and $\epsilon 4$ carriers with respect to $\epsilon 3/\epsilon 3$ subjects. In the multivariate analysis, a significant association between $\epsilon 2$ carriers and neurodegenerative diseases (OR=0.59; 95% CI, 0.37–0.94; $p=0.03$) was observed (Table 2).

At the end of follow-up (range, from 1.3 to 5.2 years; median, 2.9 years), 671 patients died (32.0%; 352 men and 319 women; mean age, 81.70 ± 7.58 years; Table 3). The prevalence of mortality was associated with male gender (men 52.5% vs women 47.5%; HR=1.46; 95% CI, 1.22–1.75; $p < 0.001$), cardiovascular diseases (HR=1.31; 95% CI, 1.08–1.58; $p=0.01$), and neoplasias (HR=2.43; 95% CI,

Table 1 Clinical and functional characteristics of patients at recruitment according to apolipoprotein E (APOE) polymorphism

Characteristics	All	ApoE polymorphism ^a			
		ε3/ε3	ε4 carriers	ε2 carriers	<i>p</i>
Total typed, <i>N</i> (%)	2,099	1,531 (72.9)	346 (16.5)	222 (10.6)	–
Age (mean and SD, in years)	78.2±7.1	78.4±7.2	77.8±7.1	77.8±6.8	0.89 ^b
Gender, men %	46.5	46.2	44.2	52.7	0.12 ^c
Prevalence of disease (%)					
Cardiovascular	54.4	53.4	55.8	58.6	0.30 ^c
Neoplasias	19.7	19.9	17.6	21.6	0.48 ^c
Neurodegenerative	19.7	19.5	24.0	14.4	0.02 ^c
Prevalence of impairment (%)					
ADL	34.0	33.6	35.5	34.7	0.77 ^c
SPMSQ	22.6	21.4	46.9	23.9	0.10 ^c
CIRS-CI	95.7	95.5	95.5	97.2	0.52 ^c
MNA	54.7	54.8	56.6	51.2	0.46 ^c

Values are means with standard deviation for continuous variables and percentages for categorical variables

ADL activities of daily living, *SPMSQ* short portable mental status questionnaire, *CIRS-CI* cumulative illness rating scale-comorbidity index, *MNA* mini nutritional assessment

^a Genotype ε2/ε4 counted out (*n*=25)

^b One-way analysis of variance

^c Pearson's chi-square test

1.99–2.97; $p<0.001$), while the mortality risk was lower in elderly with neurodegenerative diseases (HR=0.65; 95% CI, 0.50–0.83; $p=0.001$). Frequency of APOE polymorphism did not differ between dead and alive (Table 3). Differences between Kaplan–Meier survival curves according to APOE genotype (ε3/ε3, ε2, and ε4 carriers) were not statically significant (log rank test, $p=0.11$).

In the Cox regression analysis, after adjustment for age, gender, concomitant diseases, functional and cognitive impairment, and nutritional status, no APOE allele was associated with all-cause mortality. Table 4 shows the results of the multivariate analysis performed on population stratified according to diseases, adjusted for age, gender, co-morbidity, malnutrition, and functional and cognitive impairment. In patients with cardiovascular diseases, carriers of the APOE ε2 allele had a significantly lower risk of mortality than the subjects with the APOE ε3/ε3 genotype (HR=0.56; 95% CI, 0.36–0.88). There were no other significant differences in the risk of all-cause mortality between APOE ε2 and ε4 carriers with respect to ε3/ε3 subjects among several stratification groups.

Discussion

In the present study, we examined the effect of APOE polymorphism on all-cause mortality in elderly hospitalized patients. The study was carried out in a relatively large series of geriatric patients recruited from the Geriatric Unit of our Institution, all Caucasians originating from Central and Southern Italy. No significant association between the APOE ε4 allele and all-cause mortality was found. Conversely, a protective effect on all-cause mortality of the APOE ε2 allele was evident in patients with cardiovascular diseases.

The effect of APOE polymorphism on mortality or human longevity has been studied extensively, but it remains a matter of debate (Panza et al. 2009). Most of the association studies on APOE polymorphism in centenarians have, up to now, used the cross-sectional design, and this kind of study is susceptible to secular changes in population genetic parameters, cohort effect, and population stratification (Panza et al. 2007). Nonetheless, several reports have provided longitudinal data concerning APOE and mortality, with inconsistent findings (Frisoni et al. 2001; Ewbank 2002; Hayden et al. 2005; Panza et al.

Table 2 Univariate and multivariate logistic regression models on clinical and functional characteristics, considered as outcomes, of patients at recruitment according to apolipoprotein E (APOE) polymorphism

Outcomes	APOE polymorphism	Univariate model		Multivariate model ^a		Also adjusted for
		OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	
Cardiovascular diseases	ε3/ε3	1		1		Neoplasias, NDD, MNA, CIRS-CI
	ε4 carrier	1.10 (0.87–1.39)	0.43	1.14 (0.87–1.50)	0.35	
	ε2 carrier	1.23 (0.93–1.64)	0.15	1.26 (0.90–1.75)	0.10	
Neoplasias	ε3/ε3	1		1		CVD, NDD, MNA
	ε4 carrier	0.86 (0.64–1.17)	0.34	0.99 (0.69–1.40)	0.94	
	ε2 carrier	1.11 (0.79–1.57)	0.54	0.98 (0.64–1.50)	0.93	
Neurodegenerative diseases	ε3/ε3	1		1		CVD, ADL, neoplasias, SPMSQ, CIRS-CI
	ε4 carrier	1.30 (0.99–1.72)	0.06	1.13 (0.81–1.58)	0.47	
	ε2 carrier	0.69 (0.47–1.03)	0.07	0.59 (0.37–0.94)	0.03	
ADL	ε3/ε3	1		1		NDD, SPMSQ, CIRS-CI, MNA
	ε4 carrier	1.09 (0.85–1.39)	0.49	0.99 (0.71–1.39)	0.96	
	ε2 carrier	1.05 (0.78–1.41)	0.75	1.23 (0.82–1.85)	0.31	
SPMSQ	ε3/ε3	1		1		Neoplasia, NDD, ADL, MNA
	ε4 carrier	1.35 (1.02–1.79)	0.04	1.28 (0.90–1.81)	0.17	
	ε2 carrier	1.15 (0.81–1.63)	0.44	1.27 (0.82–1.97)	0.29	
CIRS-CI	ε3/ε3	1		1		CVD, NDD, ADL, MNA
	ε4 carrier	1.02 (0.57–1.80)	0.95	1.16 (0.64–2.17)	0.63	
	ε2 carrier	1.63 (0.70–3.80)	0.26	1.44 (0.61–3.41)	0.40	
MNA	ε3/ε3	1		1		CVD, neoplasias, ADL, SPMSQ, CIRS-CI
	ε4-carrier	1.07 (0.84–1.37)	0.56	1.10 (0.82–1.48)	0.50	
	ε2 carrier	0.86 (0.65–1.15)	0.32	0.89 (0.62–1.28)	0.52	

CVD cardiovascular disease, NDD neurodegenerative disease, ADL activities of daily living, SPMSQ short portable mental status questionnaire, CIRS-CI cumulative illness rating scale-comorbidity index, MNA mini nutritional assessment

^a Age- and gender-adjusted

2007). Furthermore, the cohort studies carried out to date, which have looked at APOE and longevity also not in centenarian populations, tend to have short follow-up periods (typically 5 years) (Louhija et al. 2001; Bathum et al. 2006); hence, they only provide a snapshot of overall survival. Finally, although the cohort study design is expensive because of long-term follow-up, cohort studies on the oldest-old subjects can be reasonably carried out given the high mortality rate at advanced ages, and association studies using a follow-up design on nonagenarians and centenarians have already been conducted (Panza et al. 2007; Louhija et al. 2001; Bathum et al. 2006), not confirming the role of APOE polymorphism suggested in cross-sectional reports.

Although several studies reported the adverse effect of ε4 allele on survival in older people (Tilvis et al. 1998; Hayden et al. 2005) and in a wide variety of

ethnic groups (Ewbank 2007), and significant associations of the ε4 allele with mortality dependently of AD (Dal Forno et al. 2002; Rosvall et al. 2009) or cognitive impairment (Rockwood et al. 2008) have been shown, other studies reported controversial results on the relationship between this allele and longevity (Gerdes et al. 1992), or with the risk of vascular or nonvascular mortality (Panza et al. 2007). Indeed, the lack of association between the ε4 allele and increased risk of mortality reported in the present study is consistent with several other reports (Skoog et al. 1998; Koivisto et al. 2000; Frisoni et al. 2001; Slioter et al. 2001; Tschanz et al. 2004). This finding might support the hypothesis that the importance of the APOE ε4 allele as a risk factor for mortality decreases with age (Olichney et al. 1997; Panza et al. 2007). In fact, in a population of centenarians identified from the Finnish National Population Register in 1991, no

Table 3 Hazard ratios of all-cause mortality for clinical and functional characteristics of patients at baseline (follow-up: median, 2.9 years)

Characteristics	Dead N=671	Alive N=1,428	HR	95% CI	<i>p</i> ^a
Age (mean and SD, years)	81.70±7.58	79.66±6.55	1.04	1.03–1.05	≤0.001
Gender					
Female	47.5	56.2	1.00	–	Reference
Male	52.5	43.8	1.46	1.22–1.75	<0.001
APOE carriers					
ε3/ε3	75.6	71.7	1.00	–	Reference
ε2 carriers	9.4	11.1	0.79	0.58–1.07	0.13
ε4 carriers	15.1	17.2	0.81	0.63–1.04	0.10
ADL activities of daily living, CIRS-CI cumulative illness rating scale-comorbidity index, MNA mini nutritional assessment					
Cardiovascular diseases	56.3	53.4	1.31	1.08–1.58	0.01
Neoplasias	32.3	13.7	2.43	1.99–2.97	≤0.001
Neurodegenerative diseases	17.7	20.7	0.65	0.50–0.83	0.001
ADL	52.0	25.6	1.58	1.30–1.93	≤0.001
CIRS-CI	97.7	94.7	1.50	0.88–2.57	0.12
MNA	74.0	46.1	1.90	1.53–2.35	≤0.001

Data are given as mean ± SD and as percentage

ADL activities of daily living, CIRS-CI cumulative illness rating scale-comorbidity index, MNA mini nutritional assessment

^a Multivariate Cox regression model

significant survival differences at a 3-year follow-up among different APOE allele carriers was found, suggesting that in this very selected group, the protection/risk effect associated with the APOE polymorphism might be attenuated (Louhija et al. 2001). Similar results were obtained in a recent 5-year follow-up study of 1,551 Danish nonagenarians and in a cohort of 63 Spanish nonagenarians (Bathum et al. 2006; Formiga et al. 2006). Moreover, taking into consideration that the mortality risk associated with ε4 allele is modest in Europeans (Ewbank 2007), and that a decreasing trend for the ε4 allele from Northern to Southern regions of Europe in persons older than 65 years and centenarians was reported (Panza et al. 1999; Ewbank 2004), the low frequency of this allele in our population from Southern Italy could explain the lack of association between this allele and mortality.

Beyond the suggested negative effect of the ε4 allele on survival and human longevity, there is a

growing evidence of a protective effect of the ε2 allele (Kulminski et al. 2008). In fact, in the present study, in patients with cardiovascular disease, this allele appeared to be protective on all-cause mortality. The ε2 allele has been reported to reduce the risk for common geriatric diseases (Martin 1999; Eichner et al. 2002) (e.g., AD and cardiovascular disease) and related mortality in Caucasian elderly people (Lee et al. 2001). Moreover, an association of the APOE ε2 allele with aging and longevity has been also confirmed in a recent cross-sectional study of Caucasian subjects from Southern Italy, where a significant higher prevalence of ε2 allele was found in subjects 60–90 years old and in centenarians compared to younger groups (Seripa et al. 2006b). The beneficial effect of this allele was confirmed by the findings from the Kungsholmen Project, a population-based study on 1,094 individuals aged >75 years followed for 18 years, where a 28% decreased mortality risk

Table 4 Hazard ratios of all-cause mortality predicted by apolipoprotein E (APOE) in all patients and stratified by baseline disease

	APOE polymorphism ^a					
	ε2 carriers			ε4 carriers		
	HR ^b	95% CI	<i>p</i>	HR ^b	95% CI	<i>p</i>
All	0.78	0.58–1.07	0.12	0.81	0.63–1.04	0.10
Neurodegenerative diseases	0.77	0.35–1.70	0.52	0.99	0.56–1.74	0.97
Neoplasias	0.80	0.47–1.38	0.41	0.92	0.60–1.41	0.71
Cardiovascular diseases	0.56	0.36–0.88	0.01	0.82	0.59–1.14	0.23

^a ε3/ε3 reference category

^b Adjusted for age, gender, concomitant diseases, and other functional impairment

was observed for those subjects with the $\epsilon 2$ allele (Rosvall et al. 2009), although this protective effect was not found by other studies (Koivisto et al. 2000; Slioter et al. 2001; Fillenbaum et al. 2003). At present, it seems that significant differences in APOE-related mortality may be suggested, but the different study design and follow-up periods of these population-based studies may explain the different results (Panza et al. 2009). Moreover, in general, the effect of one gene is dependent on a host of factors misleading study results (i.e., genetic background, age, gender, and physiological and pathological conditions). In fact, the role of APOE on cardiovascular diseases and related mortality might be independent of lipid levels and not be mediated by low density lipoprotein or high density lipoprotein cholesterol or triglycerides per se (Mooijaart et al. 2006). In addition, cholesterol levels decline with age (Weverling-Rijnsburger et al. 1997; Abbott et al. 1998). Other findings indicate that APOE may play additional roles in the development of coronary artery disease (CAD) through macrophage cholesterol efflux, platelet aggregation, and allele-specific antioxidant and immune activities (Davignon et al. 1999; Moghadasian et al. 2001; Stannard et al. 2001). It has been speculated that the protective effect of the $\epsilon 2$ allele may be due to its hypocholesterolemic effect, but the evidence is limited (Panza et al. 2007). In fact, other researchers have found associations between $\epsilon 2$ and CAD, and between $\epsilon 2$ and hypertriglyceridemia (Panza et al. 2007). Moreover, in the Cache County study, although the sample of homozygous $\epsilon 2$ was relatively small, significantly greater risks for early mortality were shown in persons with the $\epsilon 2/\epsilon 2$ genotype (Hayden et al. 2005). Nonetheless, an association of the APOE $\epsilon 2$ allele with aging may be explained by two hypotheses. First, the progressively increased frequency of the $\epsilon 2$ allele with aging may have been a consequence of the disappearance in selected healthy participants of the $\epsilon 4$ allele, which is reported to be linked to a higher risk of age-related diseases and mortality (Ewbank 2002). Indeed, APOE $\epsilon 4$ has been defined as a frailty allele (Panza et al. 2007), and very recently, it has been associated with severe multidimensional impairment in hospitalized older patients (Pilotto et al. 2009). The second hypothesis suggests that APOE $\epsilon 2$ could be a longevity-enhancing allele, functioning independently from other APOE alleles. This hypothesis is sup-

ported by findings showing that the frequency of $\epsilon 3$ did not change with advancing age, as would be expected if the $\epsilon 2$ increase was caused by $\epsilon 4$ disappearance in the oldest age group (Seripa et al. 2006b). Indeed, an unchanged $\epsilon 3$ allele frequency across age has been reported to be an important prerequisite in carrying out genetic association studies on aging (Lewis and Brunner 2004).

Although in the present report the protective role of the $\epsilon 2$ allele was evident only in hospitalized elderly with cardiovascular diseases, we have not able to confirm the adverse effect of the $\epsilon 4$ allele and the protective effect of the $\epsilon 2$ allele for all-cause mortality in overall hospitalized elderly. However, the lack of association between APOE polymorphism and high mortality risk may be explained by a limited sample-size and a small length of follow-up. Moreover, our study was performed on a population of elderly inpatients rather than on a general older population. In this population, neoplasias were the main determinants of mortality, particularly in the women, whereas the neurodegenerative diseases had a controversial protective effect because dementia is considered a strong predictor of mortality in the elderly (Guehne et al. 2005), also regardless of APOE genotype (Little et al. 2009). Although, a study reported that dementia developing in very old age is not necessarily associated with decreased life expectancy (Franzoni et al. 1996), in our study population, the low mortality risk for this condition could be due to the short time of the follow-up. Further studies are needed to clarify the role of APOE variants on mortality in elderly patients independently of age-related diseases.

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References

- Abbott RD, Yano K, Hakim AA, Burchfiel CM, Sharp DS, Rodriguez BL, Curb JD (1998) Changes in total and high-density lipoprotein cholesterol over 10- and 20-year periods (The Honolulu Heart Program). *Am J Cardiol* 82:172–178
- Bathum L, Christiansen MSC, Bernard J, Vaupel J, McGue M, Christensen K (2006) Apolipoprotein E genotypes: relationship to cognitive functioning, cognitive decline, and survival in nonagenarians. *J Am Geriatr Soc* 54:654–658

- Corder EH, Lannfelt L, Viitanen M, Corder LS, Manton KG, Winblad B, Basun H (1996) Apolipoprotein E genotype determines survival in the oldest old (85 years or older) who have good cognition. *Arch Neurol* 53:418–422
- Dal Forno G, Carson KA, Brookmeyer R, Troncoso J, Kawas CH, Brandt J (2002) APOE genotype and survival in men and women with Alzheimer's disease. *Neurology* 58:1045–1050
- Davignon J, Cohn JS, Mabille L, Bernier L (1999) Apolipoprotein E and atherosclerosis: insight from animal and human studies. *Clin Chim Acta* 286:115–143
- De Benedictis G, Tan Q, Jeune B, Christensen K, Ukraintseva SV, Bonafè M, Franceschi C, Vaupel JW, Yashin AI (2001) Recent advances in human gene-longevity association studies. *Mech Ageing Dev* 122:909–920
- Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC (2002) Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *Am J Epidemiol* 155:487–495
- Ewbank DC (2002) Mortality differences by APOE genotype estimated from demographic synthesis. *Genet Epidemiol* 22:146–155
- Ewbank DC (2004) The APOE gene and differences in life expectancy in Europe. *J Gerontol A Biol Sci Med Sci* 59A:8–12
- Ewbank DC (2007) Differences in the association between apolipoprotein E genotype and mortality across populations. *J Gerontol A Biol Sci Med Sci* 62:899–907
- Fillenbaum GG, Burchett BM, Lee JH, Blazer DG (2003) Mortality and apolipoprotein E in African-American, and White elders: an attempted replication. *Am J Med Genet* 119A:141–146
- Formiga F, Alia P, Navarro MA, Pujol R (2006) Apolipoprotein e genotypes in nonagenarians. *J Am Geriatr Soc* 54:1471–1473
- Franzoni S, Frisoni GB, Boffeli S, Rozzini R, Trabucchi M (1996) Good nutritional oral intake is associated with equal survival in demented and nondemented very old patients. *J Am Geriatr Soc* 46:1366–1370
- Frisoni GB, Louhija J, Geroldi C, Trabucchi M (2001) Longevity and the epsilon2 allele of apolipoprotein E: the Finnish Centenarians Study. *J Gerontol A Biol Sci Med Sci* Feb 56:M75–M78
- Gerdes LA, Klausen IB, Sihm I, Færgeman O (1992) Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. *Genet Epidemiol* 9:155–167
- Guehne U, Riedel-Heller S, Angermeyer MC (2005) Mortality in dementia. *Neuroepidemiology* 25:153–162
- Guigoz Y, Vellas B (1999) The Mini Nutritional Assessment (MNA) for grading the nutritional state of elderly patients: presentation of the MNA, history and validation. *Nestle Nutr Workshop Ser Clin Perform Programme* 1:3–11
- Hayden KM, Zandi PP, Lyketsos CG, Tschanz JT, Norton MC, Khachaturian AS, Pieper CF, Welsh-Bohmer KA, Breitner JC (2005) Apolipoprotein E genotype and mortality: findings from the Cache County Study. *J Am Geriatr Soc* 53:935–942
- Herskind AM, McGue M, Holm NV, Sørensen TI, Harvald B, Vaupel JW (1996) The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum Genet* 97:319–323
- Hitt R, Young-Xu Y, Silver M, Perls T (1999) Centenarians: the older you get, the healthier you have been. *Lancet* 354:652
- Hjelmborg JV, Iachine I, Skytthe A, Vaupel JW, McGue M, Koskenvuo M, Kaprio J, Pedersen NL, Christensen K (2006) Genetic influence on human lifespan and longevity. *Hum Genet* 119:312–321
- Hsiung GY, Sadovnick AD, Feldman H (2004) Apolipoprotein E-ε4 genotype as a risk factor for cognitive decline and dementia: data from the Canadian Study of Health and Aging. *Can Med Assoc J* 171:863–867
- Iachine IA, Holm NV, Harris JR, Begun AZ, Iachina MK, Laitinen M, Kaprio J, Yashin AI (1998) How heritable is individual susceptibility to death? The results of an analysis of survival data on Danish, Swedish and Finnish twins. *Twin Res* 1:196–205
- Jofre-Monseny L, Anne-Marie Minihane A-M, Rimbach G (2008) Impact of apoE genotype on oxidative stress, inflammation and disease risk. *Mol Nutr Food Res* 52:131–145
- Katz S, Downs TD, Cash HR, Grotz RC (1970) Progress in the development of an index of ADL. *Gerontologist* 10:20–30
- Kocsis I, Vasarhelyi B, Gyorffy A, Gyorffy B (2004) Reanalysis of genotype distributions published in Neurology between 1999 and 2002. *Neurology* 63:357–358
- Koivisto AM, Lempäinen P, Koivisto K, Helkala EL, Mykkanen L, Kuusisto J, Kervinen K, Kesäniemi YA, Laakso M, Soininen H (2000) Apolipoprotein E phenotype alone does not influence survival in Alzheimer's disease: a population-based longitudinal study. *Neuroepidemiology* 19:327–332
- Kulminski AM, Ukraintseva SV, Arbeev KG, Manton KG, Oshima J, Martin GM, Il'yasova D, Yashin AI (2008) Health-protective and adverse effects of the apolipoprotein E ε2 allele in older men. *J Am Geriatr Soc* 56:478–483
- Lee JH, Tang MX, Schupf N, Stern Y, Jacobs DM, Tycko B, Mayeux R (2001) Mortality and apolipoprotein E in Hispanic, African-American, and Caucasian elders. *Am J Med Genet* 103:121–127
- Lewis SJ, Brunner EJ (2004) Methodological problems in genetic association studies of longevity—the apolipoprotein E gene as an example. *Int J Epidemiol* 33:962–970
- Licastro F, Chiappelli M, Caldarera CM, Tampieri C, Nanni S, Gallina M, Branzi A (2004) The concomitant presence of polymorphic alleles of interleukin-1β, interleukin-6 and apolipoprotein E is associated with an increased risk of myocardial infarction in elderly men. Results from a pilot study. *Mech Ageing Dev* 5:575–579
- Linn B, Linn M, Gurel L (1968) The cumulative illness rating scale. *J Am Geriatr Soc* 16:622–626
- Little DM, Crooks VC, Petitti DB, Chiu V, Schellenberg GD, Slezak JM, Jacobsen SJ (2009) Mortality, dementia, and apolipoprotein E genotype in elderly white women in the United States. *J Am Geriatr Soc* 57:231–236
- Louhija J, Viitanen M, Aguero-Torres H, Tilvis R (2001) Survival in Finnish centenarians in relation to apolipoprotein E polymorphism. *J Am Geriatr Soc* 49:1007–1008
- Mahley RW, Weisgraber KH, Huang Y (2006) Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci USA* 103:5644–5651

- Martin JB (1999) Molecular basis of the neurodegenerative disorders. *N Engl J Med* 340:1970–1980
- Moghadasian MH, McManus BM, Nguyen LB, Shefer S, Nadj M, Godin DV, Green TJ, Hill J, Yang Y, Scudamore CH, Frohlich JJ (2001) Pathophysiology of apolipoprotein E deficiency in mice: relevance to apo E-related disorders in humans. *FASEB J* 15:2623–2630
- Mooijart SP, Berbée JF, van Heemst D, Havekes LM, de Craen AJ, Slagboom PE, Rensen PC, Westendorp RG (2006) ApoE plasma levels and risk of cardiovascular mortality in old age. *PLoS Med* 3:e176
- National Institutes of Health Consensus Development Conference Statement (2003) Geriatric Assessment methods for clinical decision-making. *J Am Geriatr Soc* 51:1490–1494
- Olichney JM, Sabbagh MN, Hofstetter CR, Galasko D, Grundman M, Katzman R, Thal LJ (1997) The impact of apolipoprotein E4 on cause of death in Alzheimer's disease. *Neurology* 49:76–81
- Panza F, Solfrizzi V, Torres F, Mastroianni F, Del Parigi A, Colacicco AM, Basile AM, Capurso C, Noya R, Capurso A (1999) Decreased frequency of apolipoprotein E epsilon4 allele from Northern to Southern Europe in Alzheimer's disease patients and centenarians. *Neurosci Lett* 277:53–56
- Panza F, D'Introno A, Capurso C, Colacicco AM, Seripa D, Pilotto A, Santamato A, Capurso A, Solfrizzi V (2007) Lipoproteins, vascular-related genetic factors, and human longevity. *Rejuvenation Res* 10:441–458
- Panza F, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Seripa D, Pilotto A, Vendemiale G, Capurso A, Solfrizzi V (2009) Apolipoprotein E, dementia, and human longevity. *J Am Geriatr Soc* 57:740–742
- Perls TT, Bubrick E, Wager CG, Vijg J, Kruglyak L (1998) Siblings of centenarians live longer. *Lancet* 351:1560
- Perls T, Kunkel LM, Puca AA (2002) The genetics of exceptional human longevity. *J Am Geriatr Soc* 50:359–368
- Pfeiffer E (1975) A short portable mental status questionnaire for the assessment of organic brain deficit in elderly patients. *J Am Geriatr Soc* 23:433–441
- Pilotto A, Matera MG, Ferrucci L, Sancarlo D, Leandro G, D'Onofrio G, Seripa D, Addante F, Franceschi M, Dallapiccola B (2009) Association of apolipoprotein E and angiotensin converting enzyme gene polymorphisms with the multidimensional impairment in older patients. *Rejuvenation Res* 12:239–247
- Rockwood K, Nassar B, Mitnitski A (2008) Apolipoprotein E-polymorphism, frailty and mortality in older adults. *J Cell Mol Med* 12:2754–2761
- Rosvall L, Rizzuto D, Wang HX, Winblad B, Graff C, Fratiglioni L (2009) APOE-related mortality: effect of dementia, cardiovascular disease and gender. *Neurobiol Aging* 30:1545–1551
- Schneider S, Roessli D, Excoffier L (2000) Arlequin ver. 2.001: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland
- Seripa D, Signori E, Gravina C, Matera MG, Rinaldi M, Fazio VM (2006a) Simple and effective determination of apolipoprotein E genotypes by positive/negative polymerase chain reaction products. *Diagn Mol Pathol* 15:180–185
- Seripa D, Franceschi M, Matera MG, Panza F, Kehoe PG, Gravina C, Orsitto G, Solfrizzi V, Di Minno G, Dallapiccola B, Pilotto A (2006b) Sex differences in the association of apolipoprotein E and angiotensin-converting enzyme gene polymorphisms with healthy aging and longevity: a population-based study from Southern Italy. *J Gerontol A Biol Sci Med Sci* 61:918–923
- Skoog I, Hesse C, Aevarsson O, Landahl S, Wahlström J, Fredman P, Blennow K (1998) A population study of apoE genotype at the age of 85: relation to dementia, cerebrovascular disease, and mortality. *J Neurol Neurosurg Psychiatry* 64:37–43
- Slooter AJ, Cruts M, Van Broeckhoven C, Hofman A, van Duijn CM (2001) Apolipoprotein E and longevity: the Rotterdam Study. *J Am Geriatr Soc* 49:1258–1259
- Song Y, Stampfer MJ, Liu S (2004) Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. *Ann Intern Med* 141:137–147
- Stannard AK, Riddell DR, Sacre SM, Tagalakakis AD, Langer C, von Eckardstein A et al (2001) Cell-derived apolipoprotein E (ApoE) particles inhibit vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells. *J Biol Chem* 276:46011–46016
- Tilvis RS, Strandberg TE, Juva K (1998) Apolipoprotein E phenotypes, dementia and mortality in a prospective population sample. *J Am Geriatr Soc* 46:712–715
- Tschanz JT, Corcoran C, Skoog I, Khachaturian AS, Herrick J, Hayden KM, Welsh-Bohmer KA, Calvert T, Norton MC, Zandi P, Breitner JC, Cache County Study Group (2004) Dementia: the leading predictor of death in a defined elderly population: the Cache County Study. *Neurology* 62:1156–1162
- Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG (1997) Total cholesterol and risk of mortality in the oldest old. *Lancet* 350:1119–1123