

## Apolipoprotein E-polymorphism, frailty and mortality in older adults

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### Abstract

Although apolipoprotein E (ApoE) polymorphism is associated with variable risks of several illnesses, and with mortality, no persuasive relationship has been demonstrated with frailty. Here, the clinical examination cohort ( $n = 1452$  older adults, aged 70+ years at baseline) of the Canadian Study of Health and Aging was evaluated, with 5-year follow-up data. Frailty was defined using both the phenotypic definition from the Cardiovascular Health Study (Frailty-CHS) and the 'Frailty Index', from which age-specific trajectories of deficit accumulation can be estimated. In age-sex adjusted analyses, people with ApoE  $\epsilon 4$  allele had a higher risk of death (hazard ratio [HR] = 1.20; 95% confidence interval: 1.01–1.45), but this relationship was not significant when adjusted for cognitive impairment (1.06; 95% confidence interval: 0.88–1.27). There was no association between frailty and ApoE polymorphism, defined in age-sex adjusted models either as Frailty-CHS (ApoE4 HR 1.17; 95% confidence interval: 0.98–1.40, frailty HR 1.37; 95% confidence interval: 1.28–1.46) or by the Frailty Index (ApoE4 HR 1.07; 95% confidence interval: 0.90–1.29, frailty HR 35.3; 95% confidence interval: 20.4–61.1). The data do not support an association between ApoE polymorphism and frailty. This result did not depend on how frailty was defined.

**Keywords:** ApoE polymorphism • aging • mortality • frailty

### Introduction

Apolipoprotein E (ApoE) is a 299 amino acid plasma glycoprotein that plays a major role in lipoprotein metabolism. The ApoE gene, found on chromosome 19, has three common alleles,  $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ . ApoE allelic variation is implicated in many common illnesses. These notably include variable risks for Alzheimer's disease [1], general cognitive decline [2], coronary heart disease [3–4] and possibly other chronic conditions [5]. These illnesses, in turn, are each associated with an increasing risk of death. While the results of individual studies show some variation, a recent systematic review suggests that  $\epsilon 4/\epsilon 4$  is generally associated with a moderately high relative risk of death, and that even one copy of the  $\epsilon 4$  allele is associated with an increased mortality risk in some populations [6].

If ApoE polymorphism is associated with common, often disabling illnesses, and with mortality, we considered that it might

also be linked to frailty. This possibility has received little investigation. Although ApoE  $\epsilon 4$  has been described as a 'frailty gene' not a 'longevity gene' [7], frailty in that study was defined in relation to an observed (but otherwise unexplained) variability in the hazard rate. Our inquiry is also motivated by the desire to understand how frailty might be defined. For now, there are two somewhat different approaches to defining frailty [8]. Each definition sees frailty as a state of low reserves (of energy, physical ability and health) that jointly give rise to vulnerability [9, 10]. The so-called 'phenotypic' definition of frailty used in the Cardiovascular Health Study (Frailty-CHS) [11] defines a syndrome according to the presence of three of more specified characteristics, as detailed below. By contrast, the Frailty Index [12] counts the proportion of a much larger number of characteristics that are present in an individual without privileging some features over others. Typically, about 40 items are measured, but the range is from about 20 to more than 100 [13]. Elsewhere, we have compared the Frailty-CHS definition with a 70-item Frailty Index (14). Briefly, we showed that the definitions were moderately well correlated with each other ( $R = 0.65$ ) and that each showed higher mortality with greater frailty, but that they were not strictly comparable. In short, analyses of

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frailty should consider just how the definition of frailty might influence the results.

Here, we aim to contribute to the understanding of how ApoE variation influences health in late life. Our objectives were to determine: (1) if frailty varies by ApoE allele status, and (2) if the relationship between frailty and ApoE varies by how frailty is defined. Given that individual samples show some variability in the relationship between ApoE and mortality, we first investigated whether mortality varied by ApoE allele status in this sample.

## Materials and methods

### Study setting, population and design

The data come from the clinical examination cohort of people in the second wave of the Canadian Study of Health and Aging (CSHA-2) [9, 14, 15]. The participants in the inception cohort (CSHA-1) were a representative sample of community-dwelling ( $n = 9008$ ) and institutionalized ( $n = 1255$ ) older Canadians aged 65 and older. Those individuals who had screened positive for cognitive impairment at CSHA-1 ( $n = 350$ ) or CSHA-2 ( $n = 987$ ) were eligible for a clinical examination at CSHA-2. A score  $\leq 77$  on the Modified Mini-Mental State Examination (3MS) [16] was considered positive for cognitive impairment and people with such scores were invited to a clinical examination. Through a multi-stage process (standardized history by a nurse, clinical and neuropsychological evaluations and a case conference [17]), participants were diagnosed as having either dementia, according to DSM-III-R criteria [18] 'cognitive impairment, no dementia' – CIND [19] or no cognitive impairment (NCI). Additionally, a sample of those who screened negative on the 3MS was examined as a comparison group. While 2305 people had clinical examinations, sufficient data to reconstruct both frailty definitions, together with ApoE information, was available for only 1452.

### Calculation of the Frailty Index

The Frailty Index assumes that the more deficits that individuals accumulate, the more likely they are to be frail [13] so that instead of considering specific deficits individually, the index measures their total number [12]. Specifically, the Frailty Index is calculated as the proportion of deficits present in an individual. Its value is defined as the fraction  $x/n$ , where  $x$  is the number of deficits for a given individual, and  $n$  is the total number of variables. Each variable can be dichotomized as either '1' if the deficit is present or '0' if it is absent. The average Frailty Index for a given age is found by calculating the arithmetic mean of the individual values at that age. This approach has been cross-validated by our group in multiple samples [20], and by others [21, 22]. Here the Frailty Index was calculated from all available dichotomized variables ( $n = 70$ ) [<http://myweb.dal.ca/amitnits/CSHAclinical-variables.jpg>] [Appendix]. The items can be self-reported, or come from variables from the clinical examination, or formal tests performed with standardized scales. Note that several deficits tallied here relate to cognition.

### The Frailty-CHS definition

Elsewhere [14, 23], we have used the CSHA-2 clinical sample to compare mortality outcomes between the Frailty-CHS definition [11] and the Frailty

Index [12], using all CSHA-2 clinical examination participants (and not just those for whom ApoE allele status is known). As detailed in those reports – and as is the case here – operationalization of the Frailty-CHS definition used variables close to those employed in other phenotypic definition studies [24, 25]. *Weight loss* was defined as loss of either  $\geq 10$  pounds or  $\geq 5\%$  of body-weight in the past year. *Exhaustion* (poor endurance and energy) was based on self-report of feeling 'tired all the time'. *Low physical activity* level was operationalized as needing assistance with walking or being unable to walk. *Slowness* was defined as  $\geq 19$  sec. on the timed up and go (TUG) test [26]. *Weakness* was equated with clinically detected abnormal strength on physical examination. People were said to be 'robust' when none of the five items was present, 'frail' if three or more were present and 'pre-frail' otherwise.

### Analysis of apolipoprotein E genotypes

ApoE genotypes were identified as described elsewhere [27] using standard laboratory two methods [28, 29]. Confirmation of the results of the first analysis was done using the second method.

### Statistical analysis

After describing the sample, we analyzed differences in mortality and frailty by both ApoE gene variation and by ApoE gene dose (*i.e.* 0, 1 or 2 alleles). To determine if mortality varies by ApoE allele status (Preliminary Objective), survival curves were constructed, using Cox regression after verifying the proportionality assumption, from which hazard ratios were calculated. In this way, we evaluated the influence of age, sex and ApoE status on survival. Cox models included ApoE4 gene dose, adjusted for age and sex, with further (separate) adjustment for cognitive status. To evaluate the impact of ApoE status on frailty (Objective 1), we compared ApoE status by frailty status and further stratified by cognitive status. To see whether the result depended on how frailty was defined (Objective 2), we tested each definition separately. Specifically, the Frailty Index values were graphed against age in relation to the ApoE. The significance of differences in the Frailty Index values by ApoE genotype were estimated using ANOVA, and in distributions of the frailty index by ApoE genotype using Kolmogorov-Smirnov test. Chi-square analyses were conducted for group differences by the Frailty-CHS definition.

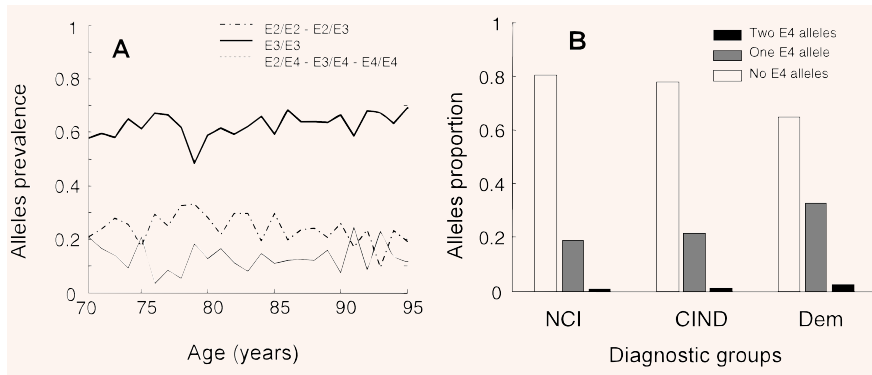
## Ethics

The CSHA protocol was approved by the research Ethics Committee of each participating institution. All participants gave written, informed consent, with additional consent for genetic testing. The proposal for secondary analyses was approved by the research Ethics Committee of the Capital District Health Authority.

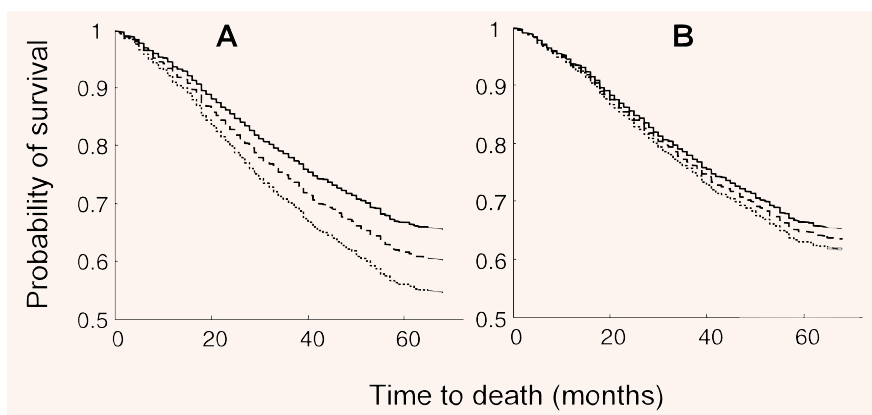
## Results

### Baseline characteristics

Of the 1452 people with ApoE genotype data, 863 (59%) were women. Over the 5 years of follow-up, 558 people died (38%) and



**Fig. 1** Characteristics of the sample. Panel **A**: age-specific prevalence of ApoE alleles. The solid line corresponds to  $\epsilon 3/\epsilon 3$  ( $n = 435$ ), the dashed line to ApoE4 ( $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 4$ ,  $\epsilon 4/\epsilon 4$ ,  $n = 127$ ) and the dot-dashed line to ApoE2 ( $\epsilon 2/\epsilon 2$  and  $\epsilon 2/\epsilon 3$ ,  $n = 89$ ). Panel **B**: Relationship between the number of ApoE  $\epsilon 4$  alleles (0,1,2) and cognitive diagnoses – No Cognitive Impairment (NCI,  $n = 651$ ), Cognitive Impairment, No Dementia (CIND,  $n = 447$ ) and dementia ( $n = 354$ ).



**Fig. 2** Survival in relation to the number of copies of the ApoE  $\epsilon 4$  allele. In both Panels, the solid line corresponds to 0 ApoE  $\epsilon 4$  alleles, the dashed line to 1 ApoE  $\epsilon 4$  allele and the dotted line to 2 ApoE  $\epsilon 4$  alleles. Panel **A**: adjusted for age and sex, the hazard ratios are significantly different from each other ( $P = 0.03$ ). Panel **B**: adjusted for age, sex and cognitive impairment, the differences are no longer statistically significant ( $P = 0.45$ ).

153 were institutionalized (11%). The mean age of the sample was 85.5 (6.9) years (women) and 83.0 (6.5) (men); most people (910; 63%) had the ApoE  $\epsilon 3/\epsilon 3$  genotype. The ApoE  $\epsilon 3/\epsilon 4$  genotype was present in 303 people (21%), and 4/4 in 16 (1%). ApoE  $\epsilon 2$  alleles were least common:  $\epsilon 2/\epsilon 2$  was observed in 13 people,  $\epsilon 2/\epsilon 3$  in 177 and  $\epsilon 2/\epsilon 4$  in 33, together comprising 15% of participants. (With respect to the ApoE  $\epsilon 4$  allele dose, 1100 [76%] had no  $\epsilon 4$  allele, 336 [23%] had 1  $\epsilon 4$  allele and 16 [1%] had 2  $\epsilon 4$  alleles.) At baseline, the age and sex of people with and without an ApoE  $\epsilon 4$  allele varied little. Women made up 58% of the ApoE4 carriers and 64% of those who were not ApoE  $\epsilon 4$  carriers. The mean age for those with ApoE  $\epsilon 4$  was 84.6 (7.4) years (women) and 82.9 (6.2) (men). At all ages, most people had the ApoE  $\epsilon 3/\epsilon 3$  genotype, with little variation in the prevalence of the various ApoE alleles by age (Fig. 1A).

Variation in ApoE alleles was most evident with respect to cognitive diagnoses. Of the 1452, 651 had NCI (45%), 447 were diagnosed with CIND (38%) and 354 with dementia (24%). Proportionately more cognitive impairment (either CIND or dementia) had at least one ApoE  $\epsilon 4$  allele, although most people with cognitive impairment did not carry the ApoE  $\epsilon 4$  allele (Fig. 1B).

## ApoE and mortality

For each genotype, people with cognitive impairment had significantly worse survival than those who did not (data not shown).

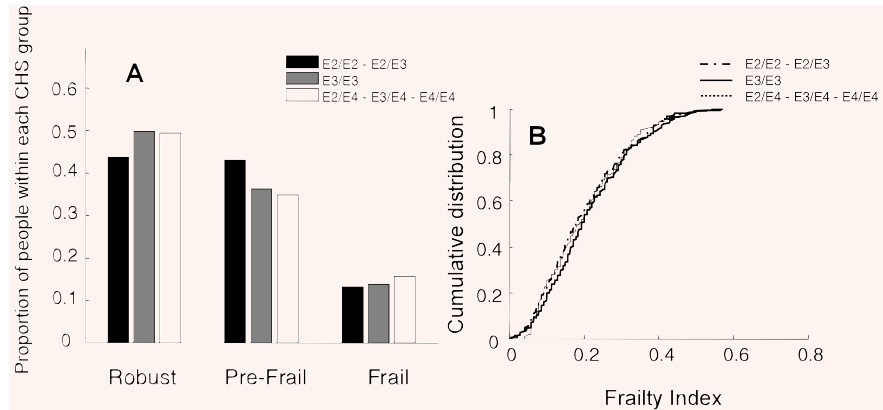
Across genotypes, people with NCI survived longest, those with dementia survived least and those with CIND had survival profiles in between the other two. In Cox regression analyses age (HR = 1.08; 95% confidence interval: 1.06–1.09) female sex (HR = 0.63; 95% confidence interval: 0.52–0.74) and cognitive impairment (HR = 1.53; 95% confidence interval: 1.36–1.69), each significantly affected survival. ApoE4 status was associated with worse survival in an age-sex adjusted Cox model (HR = 1.20; 95% confidence interval: 1.01–1.45) (Fig. 2A), but this relationship was not significant when cognitive status was included in the model (HR = 1.06; 95% confidence interval: 0.88–1.27) (Fig. 2B).

## ApoE, frailty and its definition

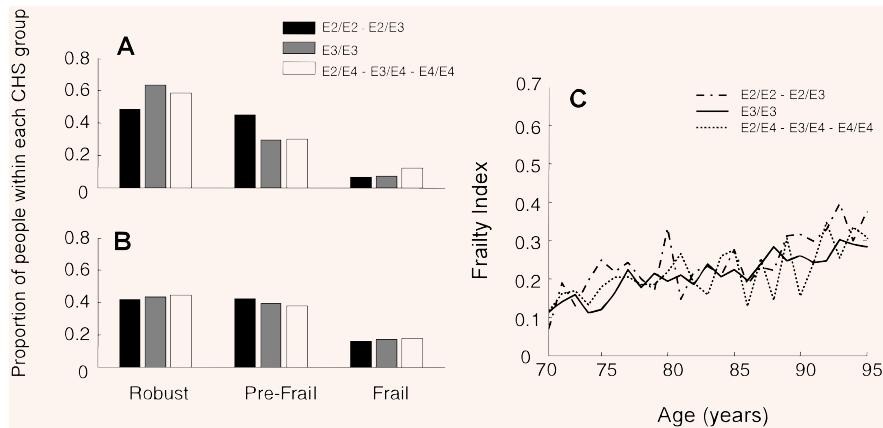
We discerned no relationship between frailty, however defined, and ApoE status (Fig. 3). Within the three Frailty-CHS groups, the differences in allelic proportions were neither significant nor was there an evident trend (Fig. 3A). Similarly, the cumulative distributions of the Frailty Index did not vary by ApoE status (Fig. 3B).

Evaluating ApoE status and frailty in relation to age also revealed no relationship (Fig. 4). By the Frailty-CHS definition, the proportions of ApoE  $\epsilon 4$  carriers and non-carriers within each group were similar for both younger (ages 70–79; Fig. 4A) and older (ages 80+, Fig. 4B) participants. Similarly, although the mean value of the Frailty Index

**Fig. 3** The relationship between ApoE alleles and frailty, according to the Frailty-CHS definition (Panel A) and the Frailty Index (Panel B).



**Fig. 4** The relationship between ApoE alleles, frailty and age, according to the Frailty-CHS definition (Panel A, for people age 70–79; Panel B, for people aged 80+) and the Frailty Index (Panel C).



increased with age, there was no difference in the change with age by ApoE status (Fig. 4C).

Given the relationship between age, sex, ApoE and mortality in relation to cognition, the relationship between genotype, frailty and cognitive impairment is of interest. For each ApoE genotype, increasing cognitive impairment was significantly associated with worse frailty, defined either by the Frailty-CHS approach (Fig. 5A) or the Frailty Index (Fig. 5B).

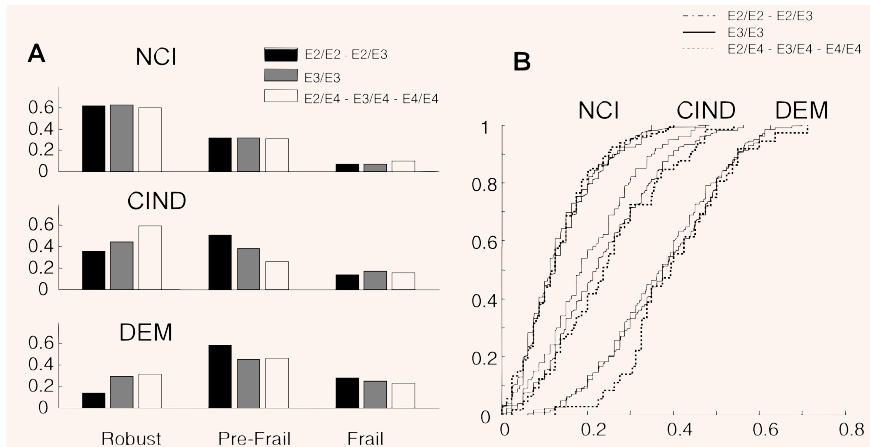
In general, the absence of a relationship between frailty and ApoE genotype was not a result of how frailty is defined (Fig. 6). People defined as robust by the CHS definition had the lowest mean frailty index scores, and those defined as frail by the CHS definition had the highest frailty index scores, but there was no significant within-frailty group difference in the distribution of the ApoE alleles.

## Discussion

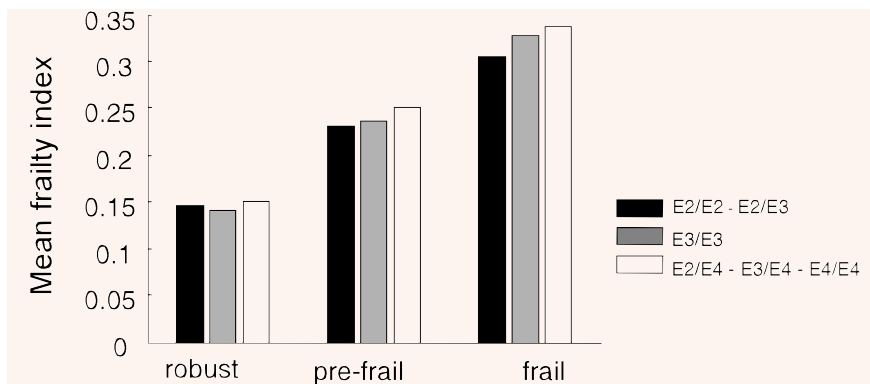
We found no association between ApoE polymorphism and frailty, whether the latter was defined according to the Frailty-CHS definition [11] or to the Frailty Index [12]. Our findings offer some sup-

port for claims of a relationship between ApoE  $\epsilon$ 4 and death [6]. Even so, the lack of significance once cognition has been taken into account makes this suspect, so that our report accords more with recent investigations of survival in relation to ApoE polymorphism and the expression of cognitive impairment [30, 31].

Still, given the considerable degree of evidence that has supported a relation between the ApoE  $\epsilon$ 4 allele and mortality [6], no single study can be seen as definitive. Many factors impinge on the relationship between ApoE and mortality. For example, the failure to fully replicate the ApoE  $\epsilon$ 4-mortality association might reflect the study having been performed with people who were significantly older than those in some of the other samples, which found mortality differences between ApoE genotypes [6, 7]. On the other hand, the findings here are not too different from the lack of a statistically significant association was observed in a report in nonagenarians [32] or in the Nun study, in which the mean age was 84 [31]. In The Cache County Study, adjustment for AD reduced the risk of death for ApoE  $\epsilon$ 3/4 (HR = 1.13, 95% confidence interval = 0.99–1.30) and for ApoE  $\epsilon$ 4/4, although the latter remained significant (HR = 1.59, 95% confidence interval: 1.15–2.14) [33]. Still, it might be that some unspecified survivor effect swamps the influence of the deleterious effect of ApoE  $\epsilon$ 4 on mortality; evidence of a mortality crossover, which is not seen



**Fig. 5** Frailty in relation to ApoE allele and the degree of cognitive impairment. Panel A shows the sample divided by cognitive status (upper third, No Cognitive Impairment, middle third, Cognitive Impairment no Dementia (CIND), and lower third dementia) according to the Frailty-CHS groupings of robust, pre-frail and frail. Panel B shows the cumulative distributions of the Frailty Index values in relation to the degree of cognitive impairment and ApoE genotype.



**Fig. 6** ApoE genotype in relation to mean frailty index values, cross-classified by the CHS frailty definition groups.

here, would support that. Such an effect, however, would have to be present for many years earlier to the mean age of 85 here, given that there was no association with deficit accumulation over any of the ages observed here. Equally, if an unspecified survivor effect were to obtain, the putative survival mechanism would appear to have to operate without regard to deficit accumulation, an intriguing possibility, but one that would go against late-life survival of the fittest [34].

Another reason to interpret our data with caution is that this population-based sample was taken from participants of the CSHA. The CSHA was designed to study dementia, so that people with dementia are relatively over-represented, making up 516 of the 1452 participants. As expected, their Frailty Index values were slightly higher (mean FI = 0.25 [0.13] versus mean FI = 0.21 [0.12] for the 936 without dementia [ $t = 5.76$ ,  $P < 0.0001$ ]). While further adjustment for both cognitive status and Frailty Index scores did not change the lack of association, adjusting for a factor that lies on the causal pathway might represent over-adjustment, another consideration in support of being cautious in interpretation. Similarly, while the focus here on all-cause ill health and mortality is indifferent to why people did not enjoy full health, a more mechanistic account would need to consider such effects. A more homogenous sample than ours might also allow the effects of specific phenotypes on specific disabilities, such as the ApoE e2

allele being present in men, but not women with instrumental activities of daily living dependency [35], or the link between ApoE4 and mobility impairment to be replicated [36].

Further considering the unrepresentativeness of the cohort, response bias must be considered. People who took part in genotyping tended to be healthier than those who were not genotyped (mean frailty index was 0.22 [0.12] for those 1452 who were genotyped, and 0.24 [0.13] for those who did not). If genotype gives rise to systematic differences in participation, this would bias the results. Finally, as this is a secondary analysis, the study does not have all potentially relevant variables. For example, there were no measurements of cholesterol, for which low levels have been associated with mortality in non-demented elderly people, and even proposed as a surrogate marker of frailty [37].

These results are of interest in relation to a report that evaluated the association of ApoE genotype on baseline cognitive functioning, cognitive decline and 5-year survival in a cohort of nonagenarians. They found no significant survival differences between ApoE genotypes [32], with perhaps the exception of people 'whose functioning was relatively well preserved' in whom the HR for e4 was 1.11 (95% confidence interval: 0.99–1.25). Similarly, a Greek study found that healthy active individuals aged 80 years and older were significantly less likely to have the ApoE4 genotype than were controls (Greek adults, median age 43; 3.1% versus 8.6%,  $P = 0.02$ ) [38]. Here,



however, the Frailty Index distributions did not show a difference at lower values of the FI (*i.e.* for healthier people) suggesting that such an effect is not present in this sample.

This study perhaps best contributes to understanding how to define frailty. Here, two markedly different approaches to defining frailty can be compared. One defines frailty specifically [11], the other emphasizes vulnerability arising from multiple problems [12]. Notably different between the two is how they handle cognition. The Frailty-CHS definition specifically excludes cognitive impairment (although whether 'exhaustion' has a cognitive component is debatable). The Frailty Index does not exclude cognitive variables. Even so, neither approach found an increased risk of frailty in patients with an ApoE  $\epsilon$ 4 allele, which suggests that it is not a 'frailty gene' in this population. This is intriguing; there are worse things than genetic effects, which increase mortality but not frailty. Such a result requires further replication before any conclusion can be drawn, and may be particularly relevant only for older, not younger samples.

How likely is it that a single gene would give rise to a state of non-specific vulnerability? It is clear both that single-gene disorders can have many manifestations and that various ApoE mutations can have pleiotropic effects [1–7]. More plausible than a single gene

giving rise to frailty, however, is that explaining additional variance in late-life health status is likely to require a polygenic approach [39]. For example, in late-onset coronary heart disease, the role of ApoE  $\epsilon$ 4 appears to be played out in association with other genetic variants, such as peroxisome proliferators activated receptor  $\gamma$ 2 Pro12 allele and endothelial nitric oxide synthase T-786C mutation [40]. For now, while these data suggest some relationship between the ApoE  $\epsilon$ 4 allele and mortality, there is no evident relationship between frailty and ApoE polymorphism.

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**Appendix:** List of variables used by the Canadian Study of Health and Aging to construct the 70-item CSHA Frailty Index from the Clinical Examination

Changes in everyday activities	Mood problems	Seizures, partial complex
Head and neck problems	Feeling sad, blue, depressed	Seizures, generalized
Poor muscle tone in neck	History of depressed mood	Syncope or blackouts
Bradykinesia, facial	Bradykinesia, limbs	Headache
Problems getting dressed	Depression (clinical impression)	Cerebrovascular problems
Problems with bathing	Sleeping changes	History of stroke
Problems carrying out personal grooming	Restlessness	History of diabetes mellitus
Urinary incontinence	Memory changes	Arterial hypertension
Toileting problems	Short-term memory impairment	Peripheral pulses
Poor muscle bulk	Long-term memory impairment	Myocardial infraction
Rectal problems	Changes in general mental functioning	Arrhythmia
Gastrointestinal problems	Onset of cognitive symptoms	Congestive heart failure
Problems cooking	Clouding or delirium	Lung problems
History of Parkinson's disease	Paranoid features	Respiratory problems
Problems going out alone	History relevant to cognitive impairment or loss	History of thyroid disease
Impaired mobility	Family history relevant to cognitive impairment or loss	Thyroid problems
Musculoskeletal problems	Impaired vibration	Malignant disease
Tired all the time	Tremor at rest	Breast problems
Abnormal muscle tone in limbs	Postural tremor	Abdominal problems
Impaired limb coordination	Intention tremor	Presence of snout reflex
Impaired coordination, trunk	Suck reflex	Palmomentary reflex
Poor standing posture	Family history of neurodegenerative disease	Other medical history
Irregular gait pattern	Falls	Cardiac problems
Skin problems		