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RESEARCH ARTICLE

Protective role of the apolipoprotein E2 allele in age-related disease traits and survival: evidence from the Long Life Family Study

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Abstract The apolipoprotein E (apoE) is a classic example of a gene exhibiting pleiotropism. We examine potential pleiotropic associations of the apoE2 allele in three biodemographic cohorts of long-living individuals, offspring, and spouses from the Long Life Family Study, and intermediate mechanisms, which can link this allele with age-related phenotypes. We focused on age-related macular degeneration, bronchitis, asthma, pneumonia, stroke, creatinine, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, diseases of heart (HD), cancer, and survival. Our analysis detected favorable associations of the ε2 allele with lower LDL-C levels, lower risks of HD, and better survival. The ε2 allele was associated with LDL-C in each gender and biodemographic cohort, including long-living individuals, offspring, and spouses, resulting in highly significant association in the entire sample ($\beta = -7.1$, $p = 6.6 \times 10^{-44}$). This allele was significantly associated with HD in long-living individuals and offspring (relative risk [RR] = 0.60, $p = 3.1 \times 10^{-6}$) but this association was not mediated by LDL-C. The protective effect on survival was specific for long-living women but it was not explained by LDL-C and HD in the adjusted model (RR = 0.70, $p = 2.1 \times 10^{-2}$). These results show that ε2 allele may favorably influence LDL-C, HD, and survival through three mechanisms. Two of them (HD- and survival-related) are pronounced in the long-living parents and their offspring; the survival-related mechanism is also sensitive to gender. The LDL-C-related mechanism appears to be independent of these factors. Insights into mechanisms linking ε2 allele with age-related phenotypes given biodemographic structure of the population studied may benefit translation of genetic discoveries to health care and personalized medicine.

Keywords ApoE · Health span · Life span

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Introduction

Insights into genetic susceptibility to age-related processes and health traits could be instructive in translational strategies on extending health span and life span (Olshansky et al. 2007; Sierra et al. 2008). Particularly, an attractive idea is to find genes, which could influence not just one health trait, but a major subset of them, and, eventually, life span (Franco et al. 2009; Martin et al. 2007). It is increasingly recognized, however, that genetic influences on complex traits, which are characteristic of post-reproductive age in modern societies, are not straightforward; the complexity is, in large part, due to the elusive role of evolution in these traits (Corella and Ordovas 2014; Kulminski 2013; Vijg and Suh 2005). For example, geriatric diseases are a relatively new phenomenon from the evolutionary viewpoint. This is because of a very modest life span of even our recent predecessors for whom, for example, world record of mean life span in 1840 was about 45 years (Oeppen and Vaupel 2002).

The problem of genetic influences on age-related human traits is complicated by at least two relevant factors. The first is the complexity of gene functions in humans, which has evolved over years to maximize fitness of individuals in different environments. In part, the problem of redundancy can be simplified by focusing on genetically more homogeneous intermediate phenotypes, often called endophenotypes (Matteini et al. 2010; Yashin et al. 2010). The second factor is the elusive role of evolution in these traits. This factor implies that "diseases are not shaped by selection," (Nesse et al. 2012), i.e., evolution did not fix the molecular basis of age-related disease traits. As a result, genetic influences on these traits can be sensitive to individuals' life course and to compositional changes in populations which often are referred to as biodemographic processes (Vaupel 2010). These two factors may interact because endogenous exposures can influence gene functions related to fitness and diseases. As a result, one should expect complex mechanisms linking genes with age-related traits, which may result in various modes of pleiotropy. In this regard, mechanisms are considered in broad sense encompassing different levels of organization starting from the micro level (e.g., genes) and ending at the macro level (e.g., phenotypic expression in specific biodemographic groups).

The apolipoprotein E (apoE) is a classic example of a pleiotropic gene, which participates in many cellular functions such as plasma lipoprotein metabolism, oxidative processes, inflammation, macrophage, glial cell and neuronal cell homeostasis, adrenal function, central nervous system physiology (Mahley and Rall 2000). While participating in these critical processes, common apoE alleles (ε2, ε3, and ε4) can contribute significantly to the pathobiology of aging and age-related traits but this contribution can be complicated by biodemographic factors discussed above (Kulminski et al. 2014, 2013).

Human epidemiological and longitudinal studies suggest that the apoE alleles affect age-related traits (Burt et al. 2008; Mahley et al. 2009; Suri et al. 2013). For example, studies suggest that the apoE2 allele might be protective against cardiovascular diseases (CVD) (Song et al. 2004), Alzheimer disease (AD) (Corder et al. 1994), and mortality (Mahley and Rall 2000). In contrast, the apoE4 allele can confer risk of Alzheimer disease (Corder et al. 1993) and decrease survival chances (Christensen et al. 2006; Kulminski et al. 2014).

The analyses of pleiotropy and mechanisms linking genes with age-related traits is important given the goal of translation of genetic discoveries into health care. Gaining insights into the connections between genes and age-related traits could help in determining population groups who are at risk for these traits. This could help us understand factors and mechanisms mediated by genes and endophenotypes and tailor disease prevention strategies and novel therapeutic approaches.

In this study, we use information on 4659 genotyped participants of the Long Life Family Study (LLFS), which includes cohorts of long-living parents, their offspring, and spouses of parents and offspring, to address two interlinked questions. First, we investigate whether the apoE2 allele can show pleiotropic effects given a broad array of age-related phenotypes measured in the same study (see "Methods" section). Second, we investigate potential role of endophenotypes in connections between the ε2 allele and downstream phenotypes in different cohorts of the LLFS population.

Methods

Data

The LLFS collected data at four field centers (three in the U.S. and one in Denmark) on families showing exceptional familial longevity. The study eligibility criteria has been described elsewhere (Pedersen et al. 2006; Sebastiani et al. 2009; Yashin et al. 2010). Briefly, in the U.S., the families eligible for the LLFS must have two living siblings aged 80+ years, two living offspring of one or more of the siblings, and a living spouse of one of the offspring. In addition, the family must demonstrate exceptional longevity based on a Family Longevity Selection Score, which is a summary-measure based on the survival experience of the oldest living generation of siblings relative to what would be expected based on birth cohort life tables (Sebastiani et al. 2009).

In Denmark, individuals who would be aged 90+ years during the study recruitment period were first identified in the Danish National Register of Persons (Pedersen et al. 2006). Then, using information on the place of birth and the names, parish registers available in regional archives were searched to locate the parents of the elderly individuals in order to identify sibships. The identified subjects were contacted to further assess the family's eligibility for participation in the LLFS using criteria parallel to that used in the U.S.

Information from the 4954 U.S. and Danish LLFS participants was collected using similar questionnaires and in-home physical examinations at baseline between 2006 and 2009. Information on onsets of diseases was assessed retrospectively at baseline from self-reports and prospectively during ongoing follow up (available currently through 2014). Biospecimens were collected at baseline. Genotyping of the apoE polymorphism was conducted using procedure detailed elsewhere (Schupf et al. 2013). The data include information on the apoE ε2/ε3/ε4 polymorphism for the 4659 LLFS participants.

Biodemographic structure of the LLFS

LLFS includes long-living (LL) individuals (N = 1384, probands and siblings), their offspring (N = 2321), and 954 spouses of long-living individuals (N = 177) and spouses of offspring (N = 777). The long-living individuals (mean age and standard deviation are 90.3 ± 6.5 years) were selected based on a history of familial longevity (see the above section). They were also subject to strong survival selection given their old age. Their offsprings were also selected based on a history of familial longevity. Unlike LL individuals and their offspring, however, the spouses were not selected based on a history of familial longevity. Given about the same young-old age of the offspring (60.5 ± 8.2 years) and spouses (65.0 ± 12.1 years), these cohorts were under about the same weak survival selection. Thus, these three groups represent three biodemographic cohorts with potentially different survival chances.

Genotypes

Genotyping of the *APOE* alleles was conducted at the Biomedical Genomics Center at the University of Minnesota using procedures detailed elsewhere (Schupf et al. 2013). In brief, genotyping of APOE was based on SNPs rs7412 and rs429358 using the Taqman genotyping platform and concordance among genotypes for both SNPs was 100 % based on blinded duplicates. We characterize the effects of the apoE2 allele (defined as **ε**2/**ε**2, **ε**2/**ε**3, or **ε**2/**ε**4 genotypes) on a number of traits contrasted by common homozygous genotype **ε**3/**ε**3. Distribution of six genotypes of the apoE locus in the entire sample of the LLFS participants as well in subsamples of the LL individuals, offspring, and spouses is given in Supplementary Table 1.

Phenotypes and endophenotypes

The focus of the study was on selected phenotypes (outcomes) and endophenotypes (intermediate phenotypes) including age-related macular degeneration (AMD), bronchitis, asthma, pneumonia, stroke, creatinine, LDL-C, high-density lipoprotein cholesterol (HDL-C), diseases of heart (HD), cancer, and death. HD included coronary heart disease, atrial fibrillation, heart failure, coronary angioplasty, or coronary artery bypass. Cancer included all sites. These traits could be sensitive to the **ε**2 allele according to prior research (more details are given in the "Discussion" section). Depending on the analyses used in the study, the same biomarker/disease could be an endophenotype or a phenotype. For example, lipids were outcomes in the analyses of their associations with the **ε**2 allele but they were endophenotypes in the analyses of the associations of this allele with diseases. Likewise, diseases were endophenotype in the analyses of the associations with risks of death.

HDL-C was measured directly in serum using the Roche HDL-Cholesterol 3rd generation direct method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). LDL-cholesterol was calculated by the Friedewald equation Creatinine was measured in serum, EDTA plasma or urine by the Roche enzymatic method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation). Information on diseases was self-reported.

ApoE-genotype-specific proportions of diseases, deaths, and mean levels of physiological markers are given in Supplementary Table 1.

Analysis

Associations of the ε2 allele with quantitative outcomes were characterized by a mixed effects linear regression model with adjustment for potential familial clustering. Measurements of lipids and creatinine were log transformed to offset potential bias due to skewness of their frequency distributions. We used information on fasting (fasted less than 8 h or 8+ h) and lipid-lowering treatment (taken lipid-lowering drugs or not) to investigate whether these conditions modulated the associations. Genotype-specific proportions of these factors are given in Supplementary Table 1.

The risks of the selected diseases and death for carriers of the ε2 allele compared with carriers of the ε3/ε3 common genotype were evaluated using the Cox proportional hazard regression model. Information on both prospective and retrospective onsets of diseases collected in the LLFS was used in these analyses. The use of retrospective onsets in a failure-type model is justified in (Prentice and Breslow 1978). These analyses provide estimates of the effects in a given population. The time variable in the Cox regression analyses was the age at onset of a trait or the age at right censoring. We used robust sandwich estimator of variances in the Cox model to adjust for familial clustering.

The analyses were conducted for men and women separately. Given differences in proportions of carriers of the apoE genotypes across family groups (i.e., LL, offspring, and spouses, see Supplementary Table 1), the analyses were conducted in each family group separately, when applicable. Because of small number of cases of AMD, asthma, bronchitis, and stroke for carriers of the ε2 allele (Supplementary Table 1), the analyses with these diseases were conducted using data for all family groups pooled together.

We evaluated potential mediating role of endophenotypes (e.g., LDL-C) in the associations of the ε2 allele with phenotypes (e.g., diseases of heart) by adjusting the models by the endophenotypes.

All statistical tests were adjusted for field centers and birth cohorts measured by age at baseline. Other adjustments were explicitly stated in the text, when applicable. Statistical analyses were conducted using SAS (release 9.3, Cary, NC, USA).

Results

The following sections provide the associations of the ε2 allele with selected biomarkers as well as with the risks of diseases and mortality highlighting significance of these associations and mediating role of endophenotypes in the downstream phenotypes. We considered: (i) lipids (LDL-C and HDL-C) and lipid-lowering therapy as endophenotypes for diseases of heart and (ii) lipids, lipid-lowering therapy and diseases as endophenotypes for mortality.

Associations of the ε2 allele with diseases of heart

For diseases of heart, we observed protective effects of the ε2 allele in long-living men and women (see Supplementary Table 2). Upon pooling data for men and women, this protective effect in the parents' generation became highly significant (Fig. 1a).

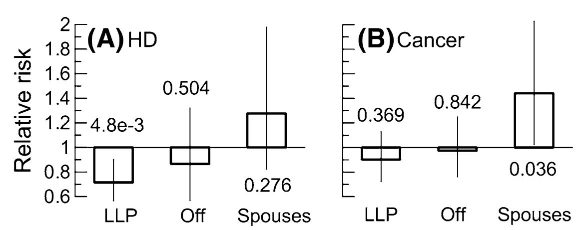

Fig. 1 Associations of the ε2 allele with the risks of **a** diseases of the heart (HD) and **b** cancer. *Bars* show relative risks for men and women combined in each family group. LLP denotes long-living parents. "Off" denotes offspring. *Vertical lines* show 95 % confidence intervals. *Numbers* show *p* values. Other details and numerical estimates are given in Supplementary Table 2

Associations of the ε2 allele with lipids

In studies of genetic associations with lipids, it was often assumed that fasting and lipid-lowering therapy might modulate genetic associations and, therefore, the analyses should be corrected for these confounding factors a priori. For example, this may be because lipid-lowering therapy may lower total cholesterol and LDL-C levels. However, while these factors might indeed be associated with lipid levels, it was actually unknown whether associations of specific genetic variants with lipids were modulated by fasting and lipid-lowering therapy. Accordingly, we first verified whether fasting and lipid-lowering therapy may modulate associations of the ε2 allele with lipids in the LLFS data.

Our analyses showed that fasting made no difference in the associations of the ε2 allele with LDL-C and high-density lipoprotein cholesterol (HDL-C) levels (Supplementary Table 3). Accordingly, fasting status was disregarded in further analyses. Lipid-lowering therapy, however, did affect the associations of the ε2 allele with LDL-C but not with HDL-C (Supplementary Table 4). For consistency, lipid-lowering therapy was retained in the model.

Table 1 showed highly significant protective associations of the ε2 allele with LDL-C showing the carriers of the ε2 allele having lower LDL-C levels. The effect sizes were remarkably consistent in each family group with somewhat larger absolute sizes in women. Overall, the association of the ε2 allele with LDL-C in the entire LLFS sample was highly significant, $\beta = -7.09$, $p = 6.6 \times 10^{-44}$.

Although the associations of the ε2 allele with HDL-C attained conventional and suggestive-effect significance in some family groups, these (and the other non-significant) effects showed opposite trends in few other family groups. Superposition of these antagonistic effects results in negligible effect of the ε2 allele on HDL-C in the entire LLFS population.

The role of lipids in the association of the ε2 allele with diseases of heart

Here we examined whether or not the protective associations of the ε2 allele with LDL-C (Table 1) mediated protective association of this allele with HD (Fig. 1a). This analysis helps in clarifying whether or not LDL-C may be in a causal pathway linking the ε2 allele with HD.

Table 2 showed that adjustment of the model for HD by LDL-C amplified protective effects between the ε2 allele and risks of HD in exceptional populations of the long-living parents and their offspring whereas this adjustment weakened the detrimental effect in a population of spouses. These results implied that LDL-C did not mediate the protective effect between the ε2 allele and HD in the exceptional populations whereas in spouses, LDL-C partly mediated (explained) the detrimental effect between the ε2 allele and HD.

Thus, Table 2 demonstrates that: (i) the protective effects of the ε2 allele were more characteristic for the exceptional population of long-living individuals and their offsprings than for spouses and (ii) different mechanisms may link the ε2 allele with HD in general and exceptional populations. Then, pooling data for the long-living individuals and their offspring, we saw an increase in the protective effect of the ε2 allele by about 22 % from RR = 0.73 ($p = 2.9 \times 10^{-3}$) to RR = 0.60 ($p = 3.1 \times 10^{-6}$) in this population (Table 2). Overall, this estimate implied that exceptional survivors carrying the ε2 allele had about 67 % lower risk of having HD compared to carriers of a common ε3/ε3 genotype at comparable levels of LDL-C.

The role of lipid-lowering therapy in associations of the ε2 allele with risks of HD

Table 3 showed that lipid-lowering therapy mediates small proportion of the protective association of the ε2 allele with HD in long-living parents (5 %) and a larger proportion in their offspring (14 %) and in spouses (17 %). This therapy explained a larger proportion of the protective effects in men (12 %) than in women (3 %) from the exceptional populations. Overall, drugs mediated about 8 % of the protective association of the ε2 allele with HD in these exceptional populations of men and women combined. The difference in the effects remained, though non-significant as evidenced by overlapping confidence intervals.

Associations of the ε2 allele with the risks of death and the role of endophenotypes

Protective effects of the ε2 allele on survival were observed in male offspring, male spouses, long-living

Table 1 The associations of the ε2 allele with lipids

Outcome	Sex	Family group	N	β*	SE	p value
LDL-C	Men	LLP	557	−6.89	1.28	2.4E−07
		Offspring	758	−6.36	1.00	4.6E−10
		Spouses	333	−6.49	1.56	5.9E−05
	Women	LLP	622	−8.14	1.38	1.2E−08
		Offspring	1064	−6.97	0.88	1.5E−14
		Spouses	380	−7.42	1.65	1.4E−05
	M&W	All	3714	−7.09	0.50	6.6E−44
HDL-C	Men	LLP	559	2.46	1.24	4.8E−02
		Offspring	772	0.89	1.05	3.9E−01
		Spouses	340	−2.84	1.56	7.1E−02
	Women	LLP	625	−1.73	1.19	1.5E−01
		Offspring	1068	0.00	0.85	1.0E+00
		Spouses	381	1.73	1.54	2.6E−01
	M&W	All	3745	0.07	0.47	8.8E−01

β is the estimate of the associations of the ε2 allele with lipids in mixed effects regression model; SE denotes standard error; N denotes sample size. Lipids were represented by low density lipoprotein cholesterol (LDL-C, mg/dL) and high-density lipoprotein cholesterol (HDL-C, mg/dL)

The model was adjusted for field centers, birth cohorts, and lipid-lowering therapy, as well as for sex and family groups, when applicable

M & W is men and women, *All* denotes all family groups combined, *LLP* denotes long living parents

* The effect size beta is evaluated for 100 × $\log_{10}$(lipids)

women, and female spouses (Supplementary Table 5). However, marginal significance was attained only in a relatively small sample size of male spouses (RR = 0.33, $p = 0.064$).

Having observed strong protective effects of the ε2 allele on LDL-C (Table 1) and HD (Table 2), as well as modulating role of lipid-lowering drugs (Table 3), we examined whether or not these factors could modulate the associations of the ε2 allele with risks of deaths. Table 4 showed that LDL-C (Model 2) played an important modulating role improving the protective association of the ε2 allele with risks of death in long-living women. This association attained conventional significance largely due to improving the effect size by about 17 % from RR = 0.83 ($p = 0.208$) to RR = 0.71 ($p = 0.022$). Protective effect was also improved in male spouses (Table 4).

HD and lipid-lowering therapy showed minor effect on the associations of the ε2 allele with risks of death (Table 4, compare Models 2 and 3). Other diseases used in the analyses did not affect the estimates (Supplementary Table 6).

Associations of the ε2 allele with cancer

Interestingly, the ε2 allele was found to be adversely associated with risks of cancer in male and female spouses (Supplementary Table 2). Pooling data for men and women, detrimental effect in spouses attained conventional significance (Fig. 1b). Figure 1 shows that protective effects of the ε2 allele (regardless of their significance) against cancer and HD were consistently observed in exceptional population of long living parents and their offspring. Detrimental effects (again, regardless of their significance) were also consistently seen in the spouses.

Moderate associations of the ε2 allele with pneumonia, stroke, and creatinine levels

Marginally significant protective association with risk of stroke was observed in men (RR = 0.55, $p = 0.055$) but not in women (RR = 1.31, $p = 0.29$) (Supplementary Table 7).

Table 2 Associations of the ε2 allele with the risks of diseases of the heart: the role of LDL-C

Sex	Family group	N_{total}	N_{event}	Model 1			Model 2		
				RR	95 % CI	*p* value	RR	95 % CI	*p* value
Men	LLP	550	267	0.75	0.55–1.01	5.9E−02	0.61	0.45–0.83	1.5E−03
	Offspring	752	75	0.69	0.38–1.25	2.2E−01	0.54	0.31–0.97	4.0E−02
	Spouses	331	59	1.47	0.82–2.62	2.0E−01	1.32	0.73–2.41	3.6E−01
	LLP + offspring	1302	342	0.72	0.55–0.94	1.5E−02	0.58	0.45–0.76	7.8E−05
Women	LLP	597	213	0.70	0.48–1.03	7.2E−02	0.59	0.40–0.89	1.1E−02
	Offspring	1063	53	0.90	0.45–1.82	7.8E−01	0.79	0.39–1.63	5.3E−01
	Spouses	379	45	1.23	0.60–2.51	5.7E−01	1.08	0.53–2.19	8.4E−01
	LLP + offspring	1660	266	0.74	0.53–1.04	7.9E−02	0.63	0.44–0.89	8.7E−03
M&W	LLP	1147	480	0.73	0.57–0.92	7.5E−03	0.60	0.47–0.76	3.5E−05
M&W	Offspring	1815	128	0.78	0.50–1.22	2.7E−01	0.63	0.40–1.00	4.7E−02
M&W	Spouses	710	104	1.31	0.84–2.06	2.4E−01	1.17	0.74–1.84	5.0E−01
M&W	LLP + offspring	2962	608	0.73	0.59–0.90	2.9E−03	0.60	0.49–0.74	3.1E−06

RR is the relative risk evaluated using the Cox proportional hazard regression model, *CI* denotes confidence interval, *LLP* denotes long living parents

Model 1 basic adjustment (field center, birth cohorts, as well as sex and family groups, when applicable), *Model 2* basic adjustment + LDL-C

The results of Model 1 are not the same as in Fig. 1a because individuals with missing information on LDL-C (3.5 %) were excluded from these analyses

N_{total} is the sample size. N_{event} is the number of events

Table 3 Associations of the ε2 allele with the risks of HD: the role of lipid-lowering therapy

Sex	Family group	N_{total}	N_{event}	Model 1			Model 2		
				RR	95 % CI	*p* value	RR	95 % CI	*p* value
M&W	LLP	1147	480	0.60	0.47–0.76	3.5E−05	0.63	0.49–0.81	2.3E−04
M&W	Offspring	1815	128	0.63	0.40–1.00	4.7E−02	0.72	0.46–1.14	1.6E−01
M&W	Spouses	710	104	1.17	0.74–1.84	5.0E−01	1.37	0.85–2.20	1.9E−01
Men	LLP + offspring	1302	342	0.58	0.45–0.76	7.8E−05	0.65	0.49–0.85	1.7E−03
Women	LLP + offspring	1660	266	0.63	0.44–0.89	8.7E−03	0.65	0.46–0.93	1.7E−02
M&W	LLP + offspring	2962	608	0.60	0.49–0.74	3.1E−06	0.65	0.52–0.80	8.6E−05

RR is the relative risk evaluated using the Cox proportional hazard regression model, *CI* denotes confidence interval, *LLP* denotes long living parents; *M&W* denotes men and women

Model 1 basic adjustment (field centers, birth cohorts, as well as sex and family groups, when applicable) + LDL-C, *Model 2* basic adjustment + LDL-C + lipid-lowering therapy

N_{total} is the sample size, N_{event} is the number of events

The risks of pneumonia for carriers of the ε2 allele were mixed across sexes and family groups (Supplementary Table 7). Detrimental suggestive-effect association (RR = 1.53, $p = 0.088$) was observed in male spouses. Trend on protective effect of the ε2 allele was observed in long living men (RR = 0.77, $p = 0.16$).

Similarly, the associations of the ε2 allele with creatinine levels were mixed across sexes and family groups (Supplementary Table 8). The ε2 allele seemed to be associated with decreased creatinine levels in male spouses ($\beta = -2.09$, $p = 0.031$) and, in female offspring ($\beta = -1.06$, $p = 0.058$).

Table 4 Associations of the ε2 allele with the risks of death: the role of endophenotypes

Sex	Family group	N_{total}	N_{event}	Model 1			Model 2			Model 3		
				RR	95 % CI	*p* value	RR	95 % CI	*p* value	RR	95 % CI	*p* value
Men	LLP	556	301	1.21	0.93–1.59	0.163	1.14	0.87–1.48	0.355	1.10	0.84–1.44	0.492
	Offspring	758	31	0.87	0.39–1.94	0.732	0.95	0.44–2.05	0.895	1.06	0.48–2.31	0.893
	Spouses	333	29	0.29	0.05–1.58	0.153	0.22	0.04–1.19	0.079	0.17	0.03–1.10	0.062
Women	LLP	622	318	0.83	0.63–1.11	0.208	0.71	0.52–0.95	0.022	0.70	0.52–0.95	0.021
	Offspring	1064	29	1.15	0.46–2.89	0.764	1.21	0.49–3.00	0.688	1.19	0.47–2.96	0.716
	Spouses	380	27	1.01	0.39–2.58	0.989	1.00	0.40–2.48	0.994	0.83	0.33–2.08	0.686

RR is the relative risk evaluated using the Cox proportional hazard regression model, *CI* denotes confidence interval, *LLP* denotes long living parents

Model 1 basic adjustment (field center and birth cohorts), *Model 2* basic adjustment + LDL-C, *Model 3* basic adjustment + LDL-C + diseases of heart + lipid-lowering therapy

The results of the Model 1 are not the same as in Supplementary Table 5 because individuals with missing information on LDL-C (3.5 %) were excluded from these analyses

N_{total} is the sample size, N_{event} is the number of events

Non-significant associations of the ε2 allele with AMD, asthma, and bronchitis

The analyses did not reveal significant associations of the ε2 allele with the risks of age-related macular degeneration (AMD), asthma, or bronchitis either for males or for females (Supplementary Table 7). The lack of significance could be a result of the sample size due to small number of disease cases.

Discussion

Allelic variations at the apoE locus have been known to influence various age-related traits such as AD, hyperlipidemia, cardiovascular disease, hemorrhagic stroke and survival. Studies in the recent past have indicated that the apoE2 allele might be protective for survival, associated with decreased levels of serum cholesterol, lower risk of cardiovascular diseases, and has neuroprotective effects against AD, infectious diseases, gastric cancer and other age-related disorders. Prior studies have also provided evidence that the apoE2 allele possesses anti-inflammatory and antioxidant properties and involved in DNA damage response and repair. Despite the evidence for a strong association of apoE2 with health span and longevity, fundamental hurdles to the translation of apoE2 into novel therapeutics include the lack of information on genotype-phenotype relationships and the poor understanding of the functional pathways involved in mediating the protective effects.

In light of these observations, we undertook the current study on the LLFS cohorts to examine two interlinked questions. First, we investigated potential roles of the apoE2 allele in an array of complex age-related traits including AMD, bronchitis, asthma, pneumonia, stroke, creatinine, LDL-C, HDL-C, HD, cancer, and survival. These traits were selected based on published reports on their association with the apoE polymorphism as discussed in sections below. Second, we examined potential mechanisms linking the ε2 allele, endophenotypes, and downstream phenotypes by analyzing modulating role of endophenotypes in the associations of the ε2 allele with phenotypes considering the biodemographic structure of the LLFS population. This structure reflects the study design in the selection of the LLFS participants (see more details in "Methods" section on "Biodemographic structure of the LLFS".

No significant effects of the ε2 allele on AMD, asthma, or bronchitis

AMD

Previously, the e4 allele was reported to have a significant association with a decreased risk of AMD and the ε2 allele was reported to be possibly associated with an increased risk of AMD (de Jong 2006;

Kulminski et al. 2008; Thakkinstian et al. 2006). Some studies report, however, suggestive-effect protective associations (Klaver et al. 1998) whereas the other show non-significant effect of the ε2 allele (Schultz et al. 2003).

Our analyses show no significant associations of the ε2 allele with AMD in the LLFS. Small number of AMD cases for the ε2 carriers does not allow us to examine whether or not the lack of the detrimental effect of the ε2 allele on AMD could be due to exceptional nature of the LLFS population.

Asthma and bronchitis

The apoE gene has been hypothesized to play a role in asthma and bronchitis based on studies on humanized apoE mice. (Yao et al. 2012). huApoE alleles differentially modified key pathogenic responses to nasal repeated house dust mite challenges, which can be stratified, in rank order of increasing disease severity, ε3 < ε4 < ε2. Compared with muApoE, the huApoE3 allele confers a protective phenotype with reduced airway hyperreactivity, mucous cell metaplasia, and airway inflammation, whereas the huApoE4 allele has an intermediate asthma phenotype with selectively attenuated AHR and serum IgE production.

However, the results of our analyses do not support connection of the ε2 allele with bronchitis and asthma in the LLFS participants. Again, small number of the disease cases prevents gaining insights on the role of exceptionality of the LLFS population.

The ε2 allele and pneumonia, stroke, and creatinine levels

Pneumonia

There is evidence that apoE could play a role in reducing susceptibility to three categories of infectious agents: viruses, bacteria, and protozoan parasites (Mahley and Rall 2000). Furthermore, the apoE gene may influence both innate and acquired immunity in a mouse model (Roselaar and Daugherty 1998). The susceptibility of apoE knock-out mice to *Klebsiella pneumoniae* and lowered inhibition of pro-inflammatory cytokine response has also been demonstrated (de Bont et al. 1999).

Our analyses suggest that, potentially, the ε2 allele can influence the risks of pneumonia in the LLFS participants. However, inconsistent effect directions in the LLFS cohorts suggest that these effects can be sensitive to such biodemographic processes as survival selection with stronger sensitivity in men.

Stroke

A meta-analysis of the associations of the apoE genotypes with stroke showed that the ε2/ε3 genotype was associated with reduced risks (Khan et al. 2013). Our analyses support this protective association but only for men carrying the ε2 allele.

Creatinine

ApoE exerts a major role in the pathogenesis and the progression of a variety of renal diseases, as well as in the atherosclerotic complications associated with them. Chronic kidney disease (CKD) as measured by estimated glomerular filtration rate (eGFR) using creatinine and cystatin is known to affect more than 30 % of older adults (Coresh et al. 2007). It is believed that development and progression of CKD is multifactorial and contributed by genetic variation, diabetes, hypertension, elevated triglycerides and low HDL-C (Hunsicker et al. 1997). There is still controversy on the effects of apoE alleles in renal diseases. Some studies have provided evidence that apoE2 allele carries a genetic risk for renal diseases whereas others have shown that the apoE4 allele is a risk factor for the progression of renal failure (Araki et al. 2003; Eto et al. 1995; Kimura et al. 1998; Oda et al. 1999). The ε4 allele is found to be associated with a lower risk of CKD in adjusted and unadjusted analyses using creatinine as biomarker (Seshasai et al. 2012). Liberopoulos et al. (Liberopoulos et al. 2004) from their studies on healthy individuals reported that individuals carrying apoE2 allele have increased levels of serum creatinine and reduced GFR compared with both ε3 and ε4 subjects.

Our results provide mixed evidence of the associations of the ε2 allele with creatinine levels across different LLFS cohorts. Significant and marginally significant associations were observed for male spouses and female offspring, respectively, meaning that the ε2 allele is associated with lower creatinine levels. These associations could be interpreted as ε2 being a favorable protective variant for kidney diseases in the LLFS cohort.

Trade-offs in the effects of the ε2 allele on HD and cancer

Diseases of heart

Two major meta-analyses of the associations of apoE with HD show trends towards a protective role of the ε2 allele (Song et al. 2004; Wilson et al. 1996). The results of our analyses, which did not focus on modulating role of endophenotypes, show that the ε2 allele can be protective against HD. A novel important insight was that this protective effect was strongly sensitive to the biodemographic factors. This means that the protective effect was pronounced in the exceptional populations of parents (RR = 0.72, $p = 4.8 \times 10^{-3}$ in men and women combined) and male offspring (RR = 0.73, $p = 0.29$) whereas in the LLFS spouses (who were not selected for chances of exceptional longevity) the effect of the ε2 allele was detrimental (RR = 1.28, $p = 0.28$).

Cancer

Evidence from prior studies on the associations of the apoE gene with cancer are conflicting and sparse with an unclear role of each allele (Anand et al. 2014). The presence of the ε2 allele was hypothesized previously to be a risk factor for cancer (Katan 1986). Some studies supported this hypothesis. Indeed, an increased risk of colon cancer for male carriers of the ε2/ε3 genotype compared to the ε3/ε3 genotype was, reported in (Watson et al. 2003). Other studies did not support that hypothesis. For example, no significant associations of the apoE genotypes with prostate cancer were reported in (Liu et al. 2015). Protective effect of the ε2 allele was reported against gastric cancer (De Feo et al. 2012).

Our results support a detrimental effect of the ε2 allele on cancer in men and women combined. However, this effect was characteristic for a sample of a general population represented by the LLFS spouses (Fig. 1b). This finding suggests that the lack of detrimental effect of the ε2 allele on cancer in the exceptional populations can be due to health-protective genetic profiles of individuals in those populations. This protective profile can explain the observed trade-off of the effects of the ε2 allele on HD and cancer seen in a sample of spouses and the exceptional populations.

The e2 allele and lipid levels

The apoE gene is known to influence lipid metabolism (Eichner et al. 2002). The apoE protein is involved in transport of cholesterol and triglycerides in blood as very low density lipoprotein and its conversion to LDL-C (Hauser et al. 2011). The ε2 allele is associated with lower cholesterol levels in most populations (Eichner et al. 2002).

Our results support these findings by showing highly significant associations of the ε2 allele with lower LDL-C levels in different populations regardless of their biodemographic structure (in populations of men and women and in populations characterized by different survival chances, Table 1). However, the ε2 allele shows mostly non-significant population-specific effects on HDL-C.

The ε2 allele, HD, LDL-C, and lipid-lowering therapy

Of the associations with the age related diseases, the ε2 allele shows the most convincing protective associations with HD. This protective effect was, however, limited to the LLFS exceptional populations. In a population of the LLFS spouses, the ε2 allele shows a trend towards adverse association (non-significant) with HD.

The ε2 allele is also found to be strongly associated with lower LDL-C levels suggesting a protection against HD (Eichner et al. 2002). These protective associations of the ε2 allele with LDL-C and HD could imply a causal pathway from ε2 to HD through LDL-C. We show that this is not the case (Table 2). In fact, we see that the protective effect of the ε2 allele on HD strengthened by 22 % (RR = 0.73 to RR = 0.60) and its significance improved by three order of magnitude ($p = 2.9 \times 10^{-3}$ to $p = 3.1 \times 10^{-6}$) in exceptional populations.

Lipid-lowering therapy tends to decrease LDL-C concentrations. Accordingly, it may contribute to causal pathways from ε2 to HD through LDL-C. Our analyses suggest that the effect of this therapy is small explaining about 8 % of the protective effect between the ε2 allele and HD in the exceptional populations

(Table 3). Accordingly, these results suggest that the lipid-lowering therapy is not a major protective factor against HD in carriers of the ε2 allele in the exceptional populations.

Thus, the ε2 allele is protective against HD in populations of the LLFS parents and their offspring, who were selected according to their chances of exceptional survival, whereas it can be unfavorable for HD in the LLFS spouses, who were not selected for exceptional survival.

Epidemiological studies suggest that long-living individuals can have biological basis to stay in good health and live long lives, although the nature of this basis remains unclear (Govindaraju et al. 2015). In part, good health and long lives can be due to favorable genetic factors (e.g., apoE, apoC3, FOXO3a, CETP, IGF genes). Partly, beneficial biological basis can be due to adaptation to specific environments [e.g., centenarians are found at a greater frequency in specific locations called "blue zones" (Poulain et al. 2013)]. The finding of antagonistic effects of the ε2 allele in populations of individuals, who were selected according to different survival chances (i.e., long-living individuals and their offspring vs. spouses) but shared the same environment, suggests that the LLFS long-living individuals and their offspring may have beneficial genetic profile (complementing the apoE2 allele) helping them in enhancing their health span (life span free of HD in the present study). Weak sensitivity of beneficial effects of the ε2 allele to lipid-lowering therapy along with the lack of favorable effect between the ε2 allele and HD in spouses indicates that this beneficial genetic profile is either weakly sensitive to lifestyle and environmental factors or it interacts differently with these exposures (Rajpathak et al. 2011).

The ε2 allele, endophenotypes, lipid-lowering therapy, and life span

Studies show that apoE can be associated with longevity and survival. The ε4 allele is typically associated with higher mortality risks whereas the ε2 allele is considered as protective (Christensen et al. 2006). Survival is a very complex phenotype which summarizes the entire life course of individuals and absorbs a variety of endophenotypes. Accordingly, if the ε2 allele is associated with endophenotypes (HD and LDL-C in this case), then, associations with survival (if any) may or may not be mediated by the associations with these endophenotypes.

We found that the ε2 allele was not significantly associated with survival in any of the LLFS cohorts, although, protective trend was seen in long-living women, male offspring, and spouses. We found, however, that LDL-C strongly moderated the effect of the ε2 allele on survival in long-living women making this protective association to attain nominal significance. This finding suggest that, similarly to the case of HD, this allele acts on LDL-C and on survival through different mechanisms. The lack of a role of other endophenotypes (HD, cancer, stroke, asthma, AMD, bronchitis, and pneumonia) and lipid-lowering therapy in the protective association of the ε2 allele with survival implies that this association is likely mediated by a third mechanism, which is independent of the LDL-C- and HD-related mechanisms.

It should be emphasized that the LLFS is a family-based cohort study examining the genetic and non-genetic factors associated with exceptional familial longevity (see "Methods" section). Long-lived individuals, their siblings and their offspring and spouses were recruited for an examination that characterized key intermediate phenotypes of longevity, including major chronic diseases, risk factors, and physical and cognitive function. The LLFS cohort is, therefore, a highly selected group of exceptional families and not representative of the overall general population. Thus caution is needed when interpreting and extrapolating these results to other populations as they have limited generalizability under the hypothesis of "pure genetic" component in complex traits.

Conclusions

Our analyses of the role of the apoE2 allele in age-related traits showed associations of this allele with many age-related traits revealing both protective and detrimental roles. ApoE2 was found to have no associations with AMD, bronchitis, and asthma in LLFS. It appears that the ε2 allele can influence the risks of pneumonia, but its effects may be sensitive to biodemographic factors. The ε2 allele shows protection against stroke (in men only) and is found to be associated with lower creatinine levels in some biodemographic groups. The ε2 allele shows trade-off effects with protective role against HD and detrimental role in cancer in the LLFS cohorts with different survival chances. The ε2 allele shows favorable associations with LDL-C, HD, and survival through three mechanisms. One mechanism (the LDL-C-related) is independent of biodemographic structure of the LLFS population. The other two (HD- and survival-related) mechanisms are distinctive of the LDL-C-related mechanism because they are sensitive to this structure, i.e., both of them are pronounced in the populations with chances of exceptional survival. The latter two mechanisms are further subdivided with the survival-related mechanism being gender sensitive whereas the HD-related mechanism is not. This implies that, potentially, favorable biomarker profile, longer health span, and longer life can be modulated by the same gene but in an independent manner. The latter implies that the effect of the ε2 allele could be modulated by other factors of genetic and/or non-genetic origin.

Our results indicate an important role of biodemographic factors in the associations of the apoE2 allele with most complex traits considered in this analysis. We believe that by reconciling contradictions on the metabolic effects of ε2 and focusing on the protective functions of apoE may open up new avenues for therapeutic target identification for healthy aging.

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Compliance with ethical standards

Conflict of interests No conflict of interests exist.

References

Anand R, Prakash SS, Veeramanikandan R et al (2014) Association between apolipoprotein E genotype and cancer susceptibility: a meta-analysis. J Cancer Res Clin Oncol 140:1075–1085

Araki S, Koya D, Makiishi T et al (2003) APOE polymorphism and the progression of diabetic nephropathy in Japanese subjects with type 2 diabetes: results of a prospective observational follow-up study. Diabetes Care 26:2416–2420

Burt TD, Agan BK, Marconi VC et al (2008) Apolipoprotein (apo) E4 enhances HIV-1 cell entry in vitro, and the APOE epsilon4/epsilon4 genotype accelerates HIV disease progression. Proc Natl Acad Sci U S A 105:8718–8723

Christensen K, Johnson TE, Vaupel JW (2006) The quest for genetic determinants of human longevity: challenges and insights. Nat Rev Genet 7:436–448

Corder EH, Saunders AM, Strittmatter WJ et al (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923

Corder EH, Saunders AM, Risch NJ et al (1994) Protective effect of apolipoprotein-E type-2 allele for late-onset Alzheimer-disease. Nat Genet 7:180–184

Corella D, Ordovas JM (2014) Aging and cardiovascular diseases: the role of gene-diet interactions. Ageing Res Rev 18:53–73

Coresh J, Selvin E, Stevens LA et al (2007) Prevalence of chronic kidney disease in the United States. JAMA 298:2038–2047

de Bont N, Netea MG, Demacker PN et al (1999) Apolipoprotein E knock-out mice are highly susceptible to endotoxemia and *Klebsiella pneumoniae* infection. J Lipid Res 40:680–685

De Feo E, Simone B, Persiani R et al (2012) A case-control study on the effect of Apolipoprotein E genotypes on gastric cancer risk and progression. BMC Cancer 12:494

de Jong PT (2006) Age-related macular degeneration. N Engl J Med 355:1474–1485

Eichner JE, Dunn ST, Perveen G et al (2002) Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 155:487–495

Eto M, Horita K, Morikawa A et al (1995) Increased frequency of apolipoprotein epsilon 2 allele in non-insulin dependent diabetic (NIDDM) patients with nephropathy. Clin Genet 48:288–292

Franco OH, Karnik K, Osborne G et al (2009) Changing course in ageing research: the healthy ageing phenotype. Maturitas 63:13–19

Govindaraju D, Atzmon G, Barzilai N (2015) Genetics, lifestyle and longevity: lessons from centenarians. Appl Transl Genomics 4:23–32

Hauser PS, Narayanaswami V, Ryan RO (2011) Apolipoprotein E: from lipid transport to neurobiology. Prog Lipid Res 50:62–74

Hunsicker LG, Adler S, Caggiula A et al (1997) Predictors of the progression of renal disease in the Modification of Diet in Renal Disease Study. Kidney Int 51:1908–1919

Katan MB (1986) Apolipoprotein E isoforms, serum cholesterol, and cancer. Lancet 1:507–508

Khan TA, Shah T, Prieto D et al (2013) Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. Int J Epidemiol 42:475–492

Kimura H, Suzuki Y, Gejyo F et al (1998) Apolipoprotein E4 reduces risk of diabetic nephropathy in patients with NIDDM. Am J Kidney Dis 31:666–673

Klaver CC, Kliffen M, van Duijn CM et al (1998) Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet 63:200–206

Kulminski AM (2013) Unraveling genetic origin of aging-related traits: evolving concepts. Rejuvenation Res 16:304–312

Kulminski AM, Ukraintseva SV, Arbeev KG et al (2008) Health-protective and adverse effects of the apolipoprotein

E epsilon2 allele in older men. J Am Geriatr Soc 56:478–483

Kulminski AM, Culminskaya I, Arbeev KG et al (2013) The role of lipid-related genes, aging-related processes, and environment in healthspan. Aging Cell 12:237–246

Kulminski AM, Arbeev KG, Culminskaya I et al (2014) Age, gender, and cancer but not neurodegenerative and cardiovascular diseases strongly modulate systemic effect of the apolipoprotein e4 allele on lifespan. PLoS Genet 10:e1004141

Liberopoulos E, Siamopoulos K, Elisaf M (2004) Apolipoprotein E and renal disease. Am J Kidney Dis 43:223–233

Liu H, Shui IM, Platz EA et al (2015) No association of ApoE genotype with risk of prostate cancer: a nested case-control study. Cancer Epidemiol Biomark Prev 24:1632–1634

Mahley RW, Rall SC Jr (2000) Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1:507–537

Mahley RW, Weisgraber KH, Huang Y (2009) Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. J Lipid Res 50(Suppl): S183–188

Martin GM, Bergman A, Barzilai N (2007) Genetic determinants of human health span and life span: progress and new opportunities. PLoS Genet 3:e125

Matteini AM, Fallin MD, Kammerer CM et al (2010) Heritability estimates of endophenotypes of long and health life: the Long Life Family Study. J Gerontol A Biol Sci Med Sci. 65:1375–1379

Nesse RM, Ganten D, Gregory TR et al (2012) Evolutionary molecular medicine. J Mol Med (Berl) 90:509–522

Oda H, Yorioka N, Ueda C et al (1999) Apolipoprotein E polymorphism and renal disease. Kidney Int Suppl 71:S25–27

Oeppen J, Vaupel JW (2002) Demography. Broken limits to life expectancy. Science 296:1029–1031

Olshansky SJ, Perry D, Miller RA et al (2007) Pursuing the longevity dividend: scientific goals for an aging world. Ann N Y Acad Sci 1114:11–13

Pedersen CB, Gotzsche H, Moller JO et al (2006) The Danish civil registration system. A cohort of eight million persons. Dan Med Bull 53:441–449

Poulain M, Herm A, Pes G (2013) The Blue Zones: areas of exceptional longevity around the world. Vienna Yearb Popul Res 1:87–108

Prentice R, Breslow N (1978) Retrospective studies and failure time models. Biometrika 65:153–158

Rajpathak SN, Liu Y, Ben-David O et al (2011) Lifestyle factors of people with exceptional longevity. J Am Geriatr Soc 59:1509–1512

Roselaar SE, Daugherty A (1998) Apolipoprotein E-deficient mice have impaired innate immune responses to Listeria monocytogenes in vivo. J Lipid Res 39:1740–1743

Schultz DW, Klein ML, Humpert A et al (2003) Lack of an association of apolipoprotein E gene polymorphisms with familial age-related macular degeneration. Arch Ophthalmol 121:679–683

Schupf N, Barral S, Perls T et al (2013) Apolipoprotein E and familial longevity. Neurobiol Aging 34:1287–1291

Sebastiani P, Hadley EC, Province M et al (2009) A family longevity selection score: ranking sibships by their longevity, size, and availability for study. Am J Epidemiol 170:1555–1562

Seshasai RK, Katz R, de Boer IH et al (2012) Apolipoprotein E and kidney function in older adults. Clin Nephrol 78:174–180

Sierra F, Hadley E, Suzman R et al (2008) Prospects for Life Span Extension. Annu Rev Med 60:457–469

Song Y, Stampfer MJ, Liu S (2004) Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 141:137–147

Suri S, Heise V, Trachtenberg AJ et al (2013) The forgotten APOE allele: a review of the evidence and suggested mechanisms for the protective effect of APOE varepsilon2. Neurosci Biobehav Rev 37:2878–2886

Thakkinstian A, Bowe S, McEvoy M et al (2006) Association between apolipoprotein E polymorphisms and age-related macular degeneration: a HuGE review and meta-analysis. Am J Epidemiol 164:813–822

Vaupel JW (2010) Biodemography of human ageing. Nature 464:536–542

Vijg J, Suh Y (2005) Genetics of longevity and aging. Annu Rev Med 56:193–212

Watson MA, Gay L, Stebbings WS et al (2003) Apolipoprotein E gene polymorphism and colorectal cancer: gender-specific modulation of risk and prognosis. Clin Sci (Lond) 104:537–545

Wilson PW, Schaefer EJ, Larson MG et al (1996) Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol 16:1250–1255

Yao X, Dai C, Fredriksson K et al (2012) Human apolipoprotein E genotypes differentially modify house dust mite-induced airway disease in mice. Am J Physiol Lung Cell Mol Physiol 302:L206–215

Yashin AI, Arbeev KG, Kulminski A et al (2010) "Predicting" parental longevity from offspring endophenotypes: data from the Long Life Family Study (LLFS). Mech Ageing Dev 131:215–222