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Estimation of Apolipoprotein E Genotype-Specific Relative Mortality Risks From the Distribution of Genotypes in Centenarians and Middle-Aged Men: Apolipoprotein E Gene Is a "Frailty Gene," Not a "Longevity Gene"

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We developed a method to estimate genotype-specific average relative mortality risk, *R*, from genotype distributions in cross-sectional studies of people belonging to different age-groups, and applied the method to new data from a study of apolipoprotein E genotypes (apoE) in 177 Danish centenarians and data from a study of 40-year-old Danish men. Twenty-one percent of the centenarians were ε2-carriers (genotypes ε2ε2 and ε3ε2) and 15% were ε4-carriers (genotypes ε4ε4 and ε4ε3) compared to 13 and 29%, respectively, of the young men. The *R*-values were 0.95 (95% CI 0.88 to 1.02) for ε2-carriers and 1.13 (95% CI 1.05 to 1.22) for ε4-carriers, using ε3ε3- and ε4ε2 genotypes as reference. Corresponding values for ε4-carriers were obtained by using published data from a French and a Finnish study of centenarians, whereas the values for ε2-carriers were about 0.90 with these data. The method to estimate mortality risk and the results associate with the view that the apoE gene is a "frailty gene." On the other hand, if odds ratios are used to summarize data from studies of this kind, they are more impressive and may propagate the misconception that apoE is a "longevity gene". Genet. Epidemiol. 19:202–210, 2000.

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INTRODUCTION

Apolipoprotein E (apoE) is centrally involved in lipoprotein metabolism but also has other important biological functions [Davignon et al., 1988; Mahley, 1988; Weisgraber et al., 1994]. The gene on chromosome 19q13.2 has three common alleles, ε2, ε3, and ε4, with varying frequencies in populations around the world [Gerdes et al., 1996]. Carriers of the ε4-allele have higher risk of coronary heart disease and Alzheimer's disease, relative to people with the most common genotype, ε3ε3, and carriers of the ε2-allele have lower risk [Davignon et al., 1988; Wilson et al., 1996; Farrer et al., 1997].

Not unexpectedly, then, the frequency of ε4-carriers is lower, and the frequency of ε2-carriers is higher in octogenarians, nonagenarians, and centenarians than in younger people [Davignon et al., 1987; Kervinen et al., 1994; Rebeck et al., 1994; Louhija et al., 1994; Schächter et al., 1994; Asada et al., 1996].

Data from studies of old people versus young people resemble data from cumulative case-control studies and the odds ratio (OR) is being used as a measure, for instance "of the relative chance of becoming a centenarian between subjects with or without a given allele" [Schächter et al., 1994], or, conversely, the risk of dying before reaching the old age. We challenge this use and interpretation of odds ratios, for the reason illustrated in Figure 1. It shows how the odds ratio calculated from the proportions of individuals in two subpopulations of a cohort aged 40 and the proportions among the survivors in the following 60 years fails to reflect that the mortality hazard in one of the subpopulations is 25% higher than in the other.

We, therefore, developed a method to obtain a different measure of association from cross-sectional genetic studies of old people versus young people, namely, estimates of the genotype-specific average relative mortality risks in the age interval considered. The method was applied to data from a study of apoE genotypes in 177 Danish centenarians.

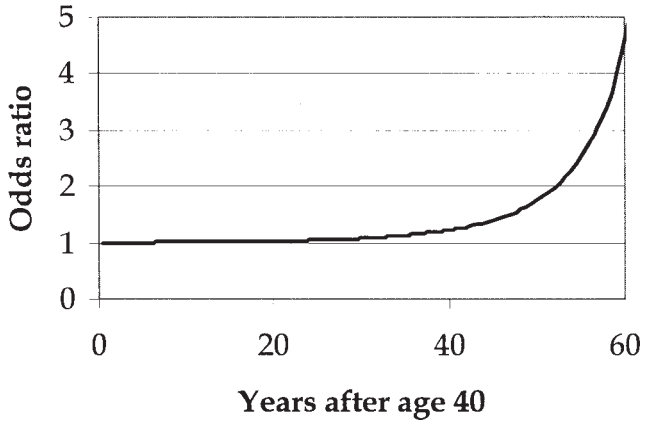

Fig. 1. The odds ratio calculated from the proportions of individuals in two subpopulations of a cohort aged 40 and the corresponding proportions among the survivors in the following 60 years, when the hazard of death in one subpopulation is 25% higher than the hazard in the other. Simulated data.

MATERIALS AND METHODS

Subjects

We approached all people over the age of 99 living in the island of Funen in May 1994 (pilot study) and all new Danish centenarians occurring in the period from April 1995 to June 1996. Fifty-eight individuals were identified in the pilot study, and 42 (72%) participated and also agreed to have blood samples taken. Twenty-seven of these were 100 years old. In the nationwide survey there were 276 eligible centenarians of which 151 (55%) participated and agreed to give a blood sample. There were no major differences between participants and non-participants in the proportion of men and women, the proportion living in nursing homes, or the proportion being severely disabled. The study was approved by the ethical committee of the County of Funen (1994) and by the Central Danish Ethical Committee (1995). The distribution of apoE genotypes in 466 randomly selected 40-year-old Danish men was used for comparison [Gerdes et al., 1992; Hansen et al., 1994].

Laboratory Methods

ApoE genotypes were determined from dried blood spots using the PCR method as described by Hixson and Vernier [Hixson and Vernier, 1990; Hansen et al., 1994].

Mathematical and Statistical Methods

ApoE allele frequencies were estimated by gene counting and 95% confidence intervals calculated as previously described [Gerdes et al., 1992].

The genotype-specific average relative mortality risk in an age interval, R, was estimated as follows. Let $\mu_1(x)$ be the hazard of death at age x in carriers of a genotype (group 1) and let $\mu_2(x)$ be the hazard in individuals without the genotype in question (group 2). Under the proportional hazard assumption $\mu_1(x) = R \cdot \mu_2(x)$, where R denotes the relative risk of group 1 compared with group 2. Let $s_1[a,b]$ and $s_2[a,b]$ denote the probabilities of survival in the two groups from some starting age a to some final age b.

Then because $s_i[a,b] = \exp(-\int_a^b \mu_i(x)dx)$, it follows that $s_1[a,b] = (s_2[a,b])^R$. Let $N(a)$ be the total size of a population at starting age a and let $p_1(a)$ and $p_2(a)$ be the proportions in the two groups. Similarly, let $N(b)$, $p_1(b)$ and $p_2(b)$ be the size, and proportions among survivors at age b, and let $S[a,b] = N(b)/N(a)$ be the probability of surviving from age a to age b in the population as a whole. Then, with $s_1[a,b]$ defined as above, the following identity holds: $N(a) \cdot p_1(a) \cdot s_1[a,b] = N(b) \cdot p_1(b)$.

Using $N(b) = N(a) \cdot S[a,b]$, rearranging terms and canceling $N(a)$ yields $s_1[a,b] = p_1(b) \cdot S[a,b]/p_1(a)$. Similarly, $s_2[a,b] = p_2(b) \cdot S[a,b]/p_2(a)$. Inserting these expressions in $s_1[a,b] = (s_2[a,b])^R$ and solving for R yields the formula we used:

$$\mathrm{R} = \frac{1\mathrm{n}\,\mathbf{p}_1(\mathbf{b}) - 1\mathrm{n}\,\mathbf{p}_1(\mathbf{a}) + 1\mathrm{n}\,\mathbf{S}[\mathbf{a},\mathbf{b}]}{1\mathrm{n}\,\mathbf{p}_2(\mathbf{b}) - 1\mathrm{n}\,\mathbf{p}_2(\mathbf{a}) + 1\mathrm{n}\,\mathbf{S}[\mathbf{a},\mathbf{b}]}.$$

The four values of p could be calculated from the data (see Table II in Results). Data from the Danish Mortality Database shows that for Danes who were 40 years old some 60 years ago, the chance of surviving from 40 to 100 (i.e., $S[a,b]$ in the formula) was about 0.002. Approximate 95% confidence intervals for R were calculated as R_L, R_U =

$R^{(1\pm1.96/\chi)}$, where χ is the square root of the null χ^2 for the observed distribution of individuals in group 1 and 2 at ages *a* and *b*. The formula was derived for rate ratios or odds ratios [Miettinen, 1985] and gave the same results with *R* as a sampling method.

RESULTS

Table I shows the distributions of apoE genotypes in Danish centenarians and in 40-year-old men. The frequency of ε2 is about 40% higher in the centenarians than in the younger and the frequency of ε4 about 40% lower.

Using individuals with ε3ε3 or ε4ε2 as a reference group we estimated the average relative mortality risks, *R*, in ε2- and ε4-carriers in the age interval from 40 to 100 years (Table II). The values indicate only modest differences in relative risks, i.e., from 0.95 in ε2-carriers to 1.13 in ε4-carriers. In contrast, the corresponding odds ratios, also shown in Table II, suggest a more dramatic impact of the apoE polymorphism.

Table III shows corresponding results calculated from published data on French and Finnish centenarians [Schächter et al., 1994; Louhija et al., 1994]. The estimated relative mortality risks in ε4-carriers are very close to the estimate based on our data, whereas the estimates in ε2-carriers tend to be lower. Again, the odds ratios are different from the values of *R*.

Figure 2 summarizes data from the present and 12 other studies on apoE allele frequencies in two or more age groups. Allele frequencies differ among populations, but the trends with increasing age appear consistent: the frequencies of ε2 and ε3 increase and the frequency of ε4 decreases.

DISCUSSION

Our study suggests that quite large differences in proportions of ε2- and ε4-carriers among middle-aged people and centenarians can be explained by modest differences in mortality risks in the centenarian's birth cohorts from about age 40

TABLE I. Distributions of Apolipoprotein E Genotypes in 177 Danish Centenarians and in 466 Randomly Selected 40-Year-Old Men*

	100-year-old							
	Women		Men		Total		40-year-old men[a]	
	n	%	n	%	n	%	n	%
ε2ε2	0	0.0	0	0.0	0	0.0	8	1.7
ε3ε2	26	19.5	11	25.0	37	20.9	54	11.6
ε3ε3	81	60.9	25	56.8	106	59.9	260	55.8
ε4ε2	5	3.8	3	6.8	8	4.5	9	1.9
ε4ε3	20	15.0	5	11.4	25	14.1	117	25.1
ε4ε4	1	0.8	0	0.0	1	0.6	18	3.9
Total	133	100.0	44	100.0	177	100.0	466	100.0

*Estimated allele frequencies in centenarians: ε2 = 12.7 (9.5–16.7), ε3 = 77.4 (72.6–81.6), ε4 = 9.9 (7.1–13.6), and in 40-year-old men: ε2 = 8.5 (6.8–10.5), ε3 = 74.1 (71.2–76.9), ε4 = 17.4 (15.0–20.0). Genotype frequencies were in Hardy-Weinberg equilibrium in both populations. Likelihood ratio χ^2 for the numbers of alleles in all centenarians and in 40-year-old men = 15.2, df = 2, $P = 0.0005$. The apoE genotype was not determined in one centenarian due to technical problems.

[a]Data from Gerdes et al. [1992] [see also Hansen et al., 1994].

TABLE II. Proportions of Apolipoprotein E Genotype Categories in 40- and 100-Year-Old Danes, and Estimated Genotype-Specific Average Relative Mortality Risks and Odds Ratios*

	Age				Relative risk - *R*		Odds ratio	
	40		100		Point	95% CI	Point	95% CI
ε2-carriers	62	13%	37	21%	0.95	(0.88–1.02)	0.71	(0.45–1.12)
ε3ε3+ε4ε2	269	58%	114	64%	1.00		1.00	
ε4-carriers	135	29%	26	15%	1.13	(1.05–1.22)	2.37	(1.42–3.94)
Total	466	100%	177	100%				

$$*R = \frac{\ln p_1(b) - \ln p_1(a) + \ln S[a,b]}{\ln p_2(b) - \ln p_2(a) + \ln S[a,b]},$$

with $p_2(b) = 0.64$, $p_2(a) = 0.58$, i.e., the proportions of ε3ε3 + ε4ε2 among centenarians and 40-year-old men, respectively. For ε2-carriers, $p_1(b) = 0.21$ and $p_1(a) = 0.13$, and for ε4-carriers, $p_1(b) = 0.15$ and $p_1(a) = 0.29$. The values of $S[a,b]$, i.e., the chance of surviving from 40 to 100 years in the Danish population, is assumed to be 0.002. If S = 0.001 *R* is 0.96 for ε2-carriers and 1.12 for ε4-carriers, respectively, and it is 0.95 and 1.15, respectively, if S = 0.004. Thus the value of S is relatively unimportant. The results were not materially influenced by whether or not the few individuals with the ε4ε2 genotype were placed in the reference group, or considered as either ε2- or ε4-carriers (not shown).

and onwards. Epsilon 2 carriers have an estimated average mortality risk that is only 4–12% lower than in ε3ε3- and ε4ε2 genotypes, and ε4-carriers has a risk that is only 10–14% higher (Tables II and III).

Is the apoE Gene a "Longevity Gene" or a "Frailty Gene"?

The measure *R* associates with the view that apoE gene is a "frailty gene" rather than a specific determinant of extreme longevity, or a "longevity gene" [van

TABLE III. Proportions of Apolipoprotein E Genotype Categories in Young and in 100-Year-Old Frenchmen and Finns, and Estimated Genotype-Specific Average Relative Mortality Risks and Odds Ratios (Calculations Based on Published Data)*

	Age				Relative risk - *R*		Odds ratio	
	27–70		100		Point	95% CI	Point	95% CI
France								
ε2-carriers	18	11%	75	23%	0.88	(0.80–0.96)	0.46	(0.27–0.81)
ε3ε3+ε4ε2	114	71%	220	68%	1.00		1.00	
ε4-carriers	29	18%	30	9%	1.10	(1.01–1.20)	2.16	(1.09–4.28)
Total	161	100%	325	100%				
	Age				Relative risk - *R*		Odds ratio	
	3–13		100		Point	95% CI	Point	95% CI
Finland								
ε2-carriers	90	6%	24	13%	0.89	(0.82–0.96)	0.49	(0.30–0.79)
ε3ε3+ε4ε2	954	60%	124	70%	1.00		1.00	
ε4-carriers	533	34%	30	17%	1.14	(1.07–1.21)	2.52	(1.61–3.93)
Total	1,577	100%	178	100%				

*Data from France are based on Schächter et al. [1994] and data from Finland are based on Louhija et al. [1994] and Lehtimaki et al. [1990]. The values of R were estimated assuming that the chance of surviving from the young age to 100 years is 0.002 in both populations.

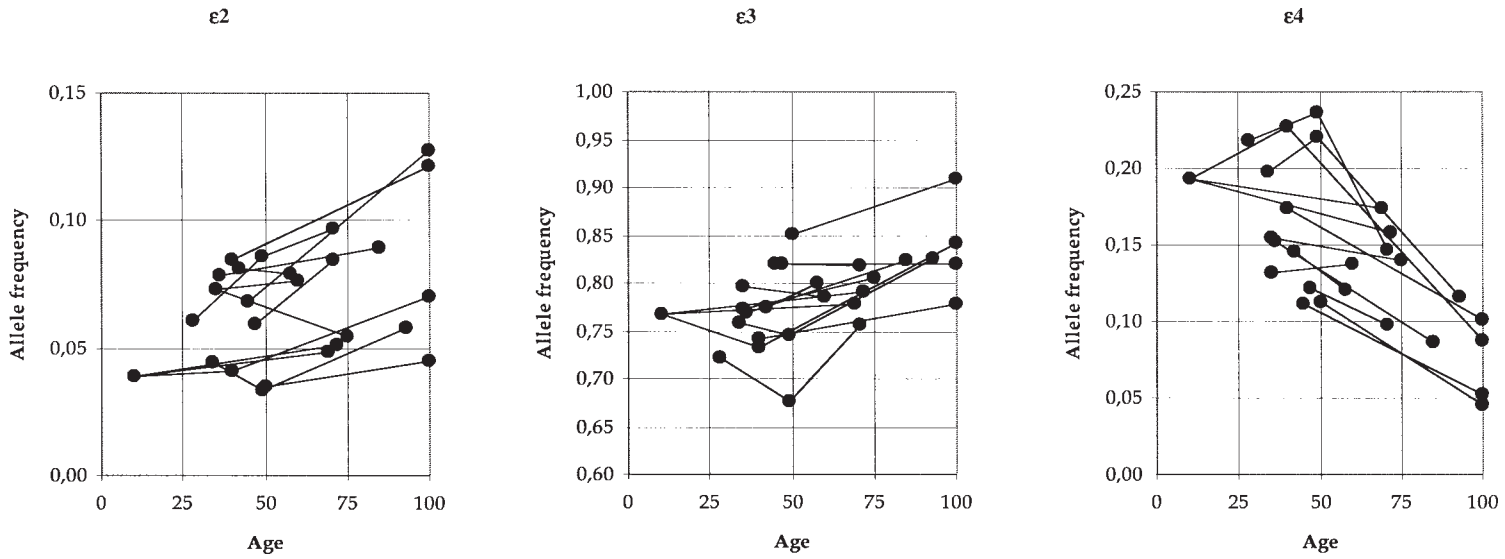

Fig. 2. Observed frequencies of the three common apolipoprotein E alleles, ε2, ε3, and ε4, in studies of population subgroups of different ages. Results from the present and 12 published studies are included [Davignon et al., 1987; Ordovas et al., 1987; Cauley et al., 1993; Eggertsen et al., 1993; Kervinen et al., 1994; Schaefer et al., 1994; Louhija et al., 1994; Schächter et al., 1994; Feskens et al., 1994; Kuusisto et al., 1995; Asada et al., 1996; Stengård et al., 1995]. Lines connect data points from corresponding subgroups by age. In studies where age groups were defined by broad ranges, we used the group's mean or median age, if reported, to plot the data, or else used the midpoint of the range.

Bockxmeer, 1994]. The latter view, we believe, is a misconception stemming in part from using odds ratios to summarize data. Odds ratios calculated from the proportions of genetic subpopulations in young and in very old people, respectively, may attain dramatic values, but fail to reflect relative mortality hazards in a meaningful way, as illustrated in Figure 1. Such odds ratios are neither comparable to odds ratios derived from density case-control study data, which consistently approximates rate ratios, nor will they approximate relative mortality risk, if data are viewed as derived from a cumulative case-control study, because the cumulated mortality risk is extremely high and the "rare disease assumption" thus grossly violated [Rothman and Greenland, 1998]. As to the latter, one cannot sustain the interpretability of odds ratios by simply inverting matters to say that it is a "rare event" to become a centenarian. The data summarized in Figure 2 support the conception of the apoE gene as a frailty gene: allele frequencies in centenarians are not peculiar, but appear as extensions of trends in data for 70-, 80-, and 90-year-olds.

Comparison of the Values of R With Results From Cohort Studies

Stengård et al. [1995, 1996] followed two cohorts of Finnish men aged 65–84 years for 5 years. About 30% of the men died and we used the published data to estimate crude genotype-specific mortality rates and rate ratios (i.e., relative mortality risks). The rate ratios for ε2-carriers with ε3ε3 genotypes as reference group were 3.5 (95%, CI 1.8–6.8) in one cohort and 1.1 (95%, CI 0.6–2.2) in the other, and for ε4-carriers they were 1.9 (95%, CI 1.2–3.1) and 1.4 (95%, CI 0.9–2.1). Hence, the values for ε2-carriers differed markedly from the *R*-value calculated in the present study (Tables II and III), and the relative mortality risk in ε4-carries were also higher.

Corder et al. [1996] determined relative mortality hazards in ε3ε2- and ε4ε3-genotypes with the ε3ε3 genotype as reference in a Swedish cohort of 1,077 men and women aged 75 or older, followed for up to 7 years [Corder et al., 1996]. Relative mortality hazards, controlling for age and sex, were 0.8 (95%, CI 0.6–1.1) in ε3ε2 genotypes and 1.3 (95%, CI 1.0–1.6) in ε4ε3 genotypes, respectively. The *R*-values in Tables II and III are not very different from these relative hazards, considering the truly different approach to obtain the values. However, the *R*-values are closer to the null hypothesis value of 1. This is perhaps not unexpected, since *R* incorporates a proportional hazard assumption and estimates average relative mortality risk over a broad age interval from about 40 to 100 years. If apoE polymorphism is not related to survival throughout the age interval, or if relative risks are changing with age, the *R*-values tend to become "diluted." The complex age-dependency of the association of apoE genotypes with Alzheimer's disease [Farrer et al., 1997] and presumably also cardiovascular disease [Haviland et al., 1995; Kuusisto et al., 1995; Zerba et al., 1996] suggest that this may very well occur.

Basic Assumption That May Not Hold

The assumption that the observed apoE genotype distribution in 40-year-old men today represents the distribution in the centenarians birth cohort some 60 years ago may not hold. Immigrations or emigrations of larger ethnic subgroups in the past can undermine such an assumption in studies conducted in some places [Stengård et al., 1995]. Also, given the multifaceted biological functions of apoE [Mahley, 1988; Mahley et al., 1990; Weisgraber et al., 1994; Ordovas et al., 1996] it cannot be ex-

cluded that genotypes have conferred differential survival under the circumstances that prevailed when today's centenarians were children, and where notably infectious diseases and nutritional disorders took a large toll.

Conclusion

The apoE gene is a frailty gene that associates with moderate differential mortality in elderly people.

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