

# Mortality Differences by APOE Genotype Estimated From Demographic Synthesis

Douglas C. Ewbank\*

*Population Studies Center, University of Pennsylvania, Philadelphia*

The 4 allele of apolipoprotein E (APOE) is associated with increased risk of two major causes of death in low-mortality populations: ischemic heart disease and Alzheimer's disease. It is less common among centenarians than at younger ages. Therefore, it is likely that it is associated with excess risk of death. This article extends demographic models that estimate relative mortality risks from changes in gene frequencies with age. The resulting demographic synthesis combines gene frequencies with data on mortality by genotype from cohort studies. The model was applied to data from Denmark, Finland, France, Italy, Sweden, and the United States. Near age 50, the 3/4 genotype is associated with a risk of death of 1.34 times that of the 3/3 (95% CI 1.18–1.67). The relative risk for 4/4 is the square of the relative risk for 3/4, 1.81. The 2/3 genotype is protective with a relative risk of 0.84 (0.68–0.93) near age 50. These relative risks move toward 1.0 at the oldest ages and APOE genotype is associated with little variation in mortality over age 100. There are no significant differences in the relative risks by sex. There is little evidence of differences within Europe in the effects of APOE. This approach can be generalized to combine data on genetic risk factors for disease from a wide variety of study designs and sample characteristics. *Genet. Epidemiol.* 22:146–155, 2002. © 2002 Wiley-Liss, Inc.

**Key words:** apolipoprotein E gene, mortality; relative risk of death; estimation methods

## INTRODUCTION

Rapid developments in genetic epidemiology have stimulated interest in identifying genes associated with survival to the oldest ages. Several studies have examined changes in gene frequencies with age for evidence of large differences in mortality by genotype [Schächter et al., 1994; Toupance et al., 1998; De Benedictis et al., 1998, 1999; Yashin et al., 1999, 2000]. Genotypes that are less frequent among centenarians are assumed to be associated with decreased chances of survival. Other studies have examined changes in gene frequencies over smaller age spans [Cauley et al., 1993; Corder et al., 1996].

Gene frequency data have generally been analyzed using standard statistical tests such as differences between proportions or logistic regressions with linear or quadratic terms for age [Farrer et al., 1997]. Recently, methods have been developed that use demographic data on the age pattern of mortality to improve these comparisons [Toupance et al., 1998; Yashin et al., 1999].

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\*Correspondence to: Douglas Ewbank, Population Studies Center, 3718 Locust Walk, Philadelphia, PA 19104.

E-mail: ewbank@pop.upenn.edu

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The model presented below combines data on longevity by genotype from multiple studies with different survey designs. Most statistical techniques are “analytical” in that they reveal components or constituent parts. In contrast, demographic synthesis uses multi-state models to combine different types of data (e.g., odds ratios and incidence rates) that provide evidence of a common phenomenon (e.g., the risk of death).

Several methods for studying mortality differences are described and applied to data on the gene for apolipoprotein-E, APOE. This gene has three common polymorphisms,  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ . The  $\epsilon 4$  allele is associated with variations in serum lipid levels [Utermann et al., 1979] and the risk of heart disease [Eichner et al., 1993; Wilson et al., 1996]. The  $\epsilon 2$  allele may protect against mortality to coronary heart disease [Sandholzer et al., 1992; Eichner et al., 1993; Wilson et al., 1996]. APOE  $\epsilon 4$  is also a major risk factor for Alzheimer’s disease [Corder et al., 1993; Farrer et al., 1997], which is the third or fourth major cause of death in the United States [Ewbank, 1999]. The  $\epsilon 2$  allele is protective against Alzheimer’s disease.

## MATERIALS AND METHODS

### Materials

Table I presents data on APOE gene frequencies by age and sex from cross-sectional studies in six countries. The largest number of studies comes from Finland, Denmark, and Sweden. Southern Europe is represented by France and Italy and the United States is represented by the Study of Osteoporotic Fractures. For the analysis, the rare  $\epsilon 2/2$  genotype is combined with the  $\epsilon 2/3$ , and the  $\epsilon 2/4$  with the  $\epsilon 3/3$ . The relative risk for the  $\epsilon 4/4$  genotype is set equal to the square of the risk associated with the  $\epsilon 3/4$  genotype. This assumption was tested (see below).

Several cohort studies provided data on survival by APOE genotype. Skoog et al. [1998] provided 3-year survival rates by genotype for a cohort of 85-year-olds in Sweden. Vogt et al. [1997] estimated the relative risk of surviving 6 years for carriers of the  $\epsilon 4$  allele for American women initially aged 65–69 and over 70. Tilvis et al. [1998] reported 5-year survival rates by genotype for cohorts aged 75, 80, and 85. These data can be combined with gene frequency data to improve the estimates of the relative risks among APOE genotypes.

### Methods

#### “Relative Risk” Method

Cross-sectional data on gene frequencies can be used to estimate relative risks using what Yashin et al. [1999] call the RR method. This method provides an estimate of the average relative risk over the age interval covered by reported gene frequencies. The proportion surviving to age  $x$  among those born in year  $T$ ,  $S(x, T)$ , is the weighted average of the survival curves for individual genotypes,  $S_i(x, T)$ :

$$S(x, T) = \sum \pi_i(0) S_i(x, T)$$

where  $\pi_i(0)$  is the proportion of the population with the  $i^{\text{th}}$  genotype at birth. The summation is over the different genotypes. The  $\pi_i(0)$  are assumed to be constant across cohorts. The proportion of those born in year  $T$  that have genotype  $i$  at age  $x$  is

$$\pi_i(x, T) = \pi_i(0) \frac{S_i(x, T)}{S(x, T)}.$$

TABLE I. Numbers of Individuals With Each APOE Genotype, Six Countries\*

Country, age group, and references	Males				Females			
	$\epsilon 2/2 + \epsilon 2/3$	$\epsilon 3/3 + \epsilon 2/4$	$\epsilon 3/4$	$\epsilon 4/4$	$\epsilon 2/2 + \epsilon 2/3$	$\epsilon 3/3 + \epsilon 2/4$	$\epsilon 3/4$	$\epsilon 4/4$
<b>Denmark</b> [Baggio et al., 1998; Gerdes et al., 2000]								
40	62	269	117	18	N.A.	N.A.	N.A.	N.A.
100	11	28	5	0	26	86	20	1
105			Sexes combined:		3	12	4	0
<b>France</b> [Schächter et al., 1994; Lambert et al., 1998]								
Middle			Sexes combined:		18	114	26	3
99+	35		6	0	258		24	0
<b>Finland</b> [Ehnhom et al., 1986; Lehtimäki et al., 1990; Kervinen et al., 1994; Tilvis et al., 1998; Frisoni et al., 2001]								
<20			Sexes combined:		90	954	483	50
20–55			Sexes combined:		43	337	196	39
21–64			Sexes combined:		15	151	82	11
75			Sexes combined:		136		46	
80			Sexes combined:		132		53	
85			Sexes combined:		134		49	
100–101	2	14 <sup>a</sup>	1	0	8	75	17	0
102–103	3	5	1	0	6	19	7	1
104–106	0	2	0	0	5	10	3	0
<b>Italy</b> [Panza et al., 1999]								
Middle			Sexes combined:		21	85	17	2
99+			Sexes combined:		16	48	3	0
90–99	2	5	1	0	6	64	16	1
<b>Sweden</b> [Eggertsen et al., 1993; Corder et al., 1996]								
<40 <sup>b</sup>			Sexes combined:		16	94	54	8
80–84	12	74	27	N.A.	34	166	78	N.A.
85–89	2	29	7	N.A.	27	95	53	N.A.
90+	4	8	2	N.A.	20	44	13	N.A.
<b>U.S.</b> [Cauley et al., 1993]								
42–50	N.A.	N.A.	N.A.	N.A.	49	320	98	6
65–90	N.A.	N.A.	N.A.	N.A.	131	592	135	12

\*N.A.: these data were not available either because the publications did not provide them or the study design did not include these groups.

<sup>a</sup>Includes one individual who is  $\epsilon 1/3$ .

<sup>b</sup>Estimated from allele frequencies assuming Hardy-Weinberg equilibrium.

The survival probability  $S(x, T)$  can be expressed in terms of age-specific mortality rates:  $S_i(x, t) = \exp[-\int \mu_i(y) dy]$ . The ratio of the mortality rate for genotype  $i$ ,  $\mu_i(x, T)$ , to the overall mortality rate,  $\mu(x, T)$ , is initially assumed to be a constant,  $r_i$ . Therefore, dropping the subscript for birth year,  $S_i(x)$  is equal to  $S_j(x)$  raised to the power  $r_i$ .

In general, we only know the life table for the whole population,  $S(x)$ . Since the overall mortality rate is a weighted average of the rates for the genotypes, we can estimate the mortality rate for the reference group as

$$\mu_1 = \frac{\mu(x)}{\pi_1 S_1(x) + \pi_2 S_2(x) r_2(0) + \dots + \pi_k S_k(x) r_k(0)}$$

Since  $S_i(x)$  is a function of  $\mu_i(x)$ , it is necessary to iterate between them, beginning with initial values for  $r_i$  of 1.0 and  $\mu_i(x)$  set to  $\mu(x)$ . The model defines the relative risks after age 40. The relative risks are assumed to change linearly from 1.0 at age 20 to  $r_i$  at age 40. The results were not sensitive to this assumption.

It is assumed that the population is in Hardy-Weinberg equilibrium so that the  $k(k+1)/2$  genotype frequencies,  $\pi_i(0)$ , are replaced by the  $k$  allele frequencies,  $f_i$ . The  $f_i$  sum to 1 and  $r_i$  is set to 1.0 as the baseline. Then there are  $(k-1)$  allele frequencies and  $k(k+1)/2$  relative risks. For APOE,  $k$  is three so that there are 7 parameters, but restrictions on the relative risks for  $\epsilon 2/2$ ,  $\epsilon 2/4$ , and  $\epsilon 4/4$  noted above reduce the number of relative risks to 4. Parameter estimates were derived by maximizing the likelihood of observing the numbers of individuals age  $x$  with each genotype from cross-sectional studies using a multinomial distribution with probabilities equal to  $S_i(x)/S(x)$ .

**Demographic Synthesis of Data From Multiple Populations**

The proposed model can be used to synthesize data from numerous studies by maximizing the joint likelihood function for all of the studies. The  $r_i$  for each sex are constrained to be constant across studies. The  $f_i$  must still be estimated for each population but they are assumed to not vary by sex. When necessary, the relative risks were calculated from the model by weighting the sex-specific survival curves.

**Adding Cohort Studies to the Synthesis**

If APOE genotype is associated with differential survival among infants and children, then declining infant and child mortality across cohorts could explain differences in gene frequencies at the oldest ages. It is possible to protect against this by including data from cohort studies of mortality by APOE genotype.

The likelihood of observing the reported proportion surviving by genotype is binomial. The estimated proportion of individuals in the age group  $x$  to  $y$  with genotype  $i$  that survives  $n$  years is equal to  $\int S_i(z+n)dz / \int S_i(z)dz$  where the integration is from  $x$  to  $y$ . The likelihood of observing the survival rates from a single study is the product of the likelihoods of observing the survival rates for the individual genotypes. These likelihood values can be combined with the likelihood values based on gene frequencies to produce a joint likelihood.

**Incorporating Changes in the Relative Risk by Age**

If there are fixed characteristics that are associated with variation in mortality, the risks observed for any one fixed characteristic will appear to decline with age. Yashin et al. [1999] proposed the ‘‘HRR’’ method of controlling for this unobserved heterogeneity by assuming a specific distribution of frailty. The following is a reformulation that emphasizes the effects of heterogeneity on the relative risks.

If frailty at age  $y$  is distributed according to a Gamma distribution with mean  $r_{i,y}$  and coefficient of variation of  $\gamma$ , then

$$\mu_{i,x} = \frac{r_{i,y} \lambda(x)}{1 + r_{i,y} \gamma^2 \int_y^x \lambda(t) dt}$$

where  $\lambda(x)$  is a reference mortality schedule for individuals with frailty of 1.0 [Manton, et al., 1986]. The risk for group  $i$  relative to a group that has a mean frailty of 1 at age 0 is:

$$r_{i,x} = r_{i,y} \frac{1 + \gamma^2 \int_y^x \lambda(t) dt}{1 + r_{i,y} \gamma^2 \int_y^x \lambda(t) dt} = r_{i,y} \left( \frac{S_{i,x}/S_{i,y}}{S_{1,x}/S_{1,y}} \right)^{\gamma^2}.$$

### Tests of Alternative Distributions of Frailty

The age pattern of change in the relative risks depends on the assumed distribution of frailty. Manton et al. [1986] provided an equation for the mean frailty of survivors to age  $x$  that encompasses both the Gamma and the Inverse Gaussian cases. The mortality rate at age  $x$  for subgroup  $i$  is:

$$\mu_{i,x} = \frac{r_{i,y} \lambda(x)}{\left( 1 + lr_{i,y} \gamma^2 \int_y^x \lambda(t) dt \right)^{1/l}}$$

where  $l$  is 1 for the Gamma distribution and 2 for the Inverse Gaussian distribution. We use this general formulation to determine how consistent the data are with either the Gamma or the Inverse Gaussian distribution of frailty. Changes in the relative risks with age are determined by the assumed value of  $l$ . For example, using the life table for Swedish females born in 1898–1902 and assuming a relative risk at age 40 of 1.20, values of  $l$  of 0.75 and 3.0 lead to relative risks of 1.13 and 0.96 at age 100. The Gamma distribution leads to a relative risk at age 100 of 1.02 and the Inverse Gaussian gives 1.10.

### Tests of Heterogeneity Among Populations

Gene-environment interactions and gene-gene interactions probably lead to differences in the relative risks across populations. Likelihood ratio tests are used to compare differences between geographic regions or by sex.

### Choice of Life Tables

Changes in the gene frequencies with age depend on the relative risks and the level of mortality. Older cohorts had higher mortality rates and will have experienced a greater drop in the frequency of risky genotypes than suggested by current life tables. It is important to test the value of using different cohort life tables for each population.

### Optimization Procedure

The data and the likelihood functions were entered into a spreadsheet program, Quattro<sup>®</sup>Pro, and maximized using a Newton-Raphson optimization routine. Numerous starting values were tested to insure identification of the global maximum.

## RESULTS

### Results From Relative Risk Method

Table II presents estimates of the relative risks of mortality for the main APOE genotypes for each of the six populations listed in Table I. The results were consistent across

**TABLE II. Estimates of the Relative Risks of Deaths at Age 40 Associated With APOE Genotypes, by Sex, Based on Cross-Section Studies of Gene Frequencies<sup>†</sup>**

Study	Estimates of the relative risks (reference is $\epsilon 3/3 + \epsilon 2/4$ except as noted)			
	Males		Females	
	$\epsilon 2/3$ & $\epsilon 2/2$	$\epsilon 3/4$	$\epsilon 2/3$ & $\epsilon 2/2$	$\epsilon 3/4$
Denmark	0.92	1.17*	0.96	1.13*
Finland	0.87	1.27*	0.90*	1.14*
France <sup>a</sup>	N.A.	1.05	N.A.	1.19*
Italy	Sexes combined:		0.95	1.26*
Sweden	0.87	1.48*	0.54*	1.20
U.S.	N.A.	N.A.	<0.20 <sup>b</sup>	1.46

<sup>†</sup>The relative risk for the  $\epsilon 4/4$  is set equal to the square of the risk for  $\epsilon 3/4$ .

N.A., there were no data available for estimating these relative risks.

<sup>a</sup>The relative risks for the study by Schächter et al. [1994], are relative to the  $\epsilon 3/3 + \epsilon 2/3 + \epsilon 2/2$  and are, therefore, probably biased upward in comparison to the estimates from the other studies.

<sup>b</sup>The estimate of the relative risk for  $\epsilon 2/3$  for U.S. females approaches zero and is significantly different from 1.0. For present purposes, the relative risk was constrained to be 0.20. This assumption does not affect the estimate of the relative risk for the  $\epsilon 3/4$ .

\*Significantly different from 1.0 at the 5% level or less.

studies. The  $\epsilon 3/4$  genotype (and by extension the  $\epsilon 4/4$  genotype) was always associated with increased risk of death, although the association was not always statistically significant (likelihood ratio tests). With the exception of the estimates for the United States (which are based on only two age groups), the relative risk for females for  $\epsilon 3/4$  ranged from 1.13 to 1.26. The range for males was slightly larger, 1.05–1.48. The estimated relative risks for the  $\epsilon 2/3$  and  $\epsilon 2/2$  genotypes were generally around 0.9 but were rarely statistically different from unity. The estimates of the allele frequencies at birth (not shown) were very similar to the reported frequencies at the younger ages.

### Results From the Demographic Synthesis

Combining all of the cross-sectional data shows that the  $\epsilon 2/3$  genotype was associated with a 9% reduction in mortality for both sexes. The risk associated with the  $\epsilon 3/4$  did not differ significantly by sex (males: 1.18, females: 1.14, combined: 1.16). The relative risk associated with  $\epsilon 4/4$  (1.39) was not significantly different from the square of the risk for  $\epsilon 3/4$ . Adding the cohort studies to the analysis led to only slight changes in the parameter estimates. This suggests that the differences in gene frequencies by age are not due to cohort differences in childhood mortality rates.

### Results From the Model Incorporating Heterogeneity

Table III presents parameter estimates based on a Gamma distribution for frailty. The  $\epsilon 2/3$  genotype again appeared to be protective (relative risk of 0.84 for sexes combined at age 40) and the  $\epsilon 3/4$  risky (1.34). Once again, the differences by sex were not statistically significant and there was no evidence for rejecting the hypothesis that the relative risk for the  $\epsilon 4/4$  is the square of that for the  $\epsilon 3/4$ .

The estimated relative risks apply at age 40. The relative risks for  $\epsilon 3/4$  and  $\epsilon 4/4$  decrease toward 1.0 increasing with age (Fig. 1) while those for the  $\epsilon 2/3$  increase toward 1.0 (not shown). At age 100, the estimated relative risks for the  $\epsilon 2/3$  and  $\epsilon 3/4$  are only 0.99 and 1.01 for Finnish males born in 1886–1890. The rate at which the relative risks change

**TABLE III. Estimates of the Relative Risks at Young Ages by APOE Genotype and Sex, Five Studies of Changes in APOE Gene Frequencies Incorporating the Effects of Unobserved Heterogeneity**

Model	$\gamma$	$\epsilon_{2/3}$ & $\epsilon_{2/2}$		$\epsilon_{3/4}$	
		Males	Females	Males	Females
By sex	0.58	0.91	0.81	1.45	1.33
No differences by sex	0.57	0.84		1.34	
(95% confidence intervals)	(0.24–0.88)	(0.68–0.93)		(1.18–1.67)	

depends on the level of mortality. For example, only 9.9% of the cohort of Finnish males born in 1886–1890 survived to age 80. In contrast, about 48.2% of Swedish females born in 1908–1910 survived to age 80. Figure 1 shows that these life tables lead to very different relative risks for  $\epsilon_{3/4}$  and  $\epsilon_{4/4}$  at the oldest ages. Similarly, the estimated relative risk for  $\epsilon_{2/3}$  at age 90 varies from 0.89 for the cohort of Swedish females to 0.95 for the cohort of Finnish males.

The estimates based on the frailty model can be compared to the previous estimates using the average relative risks over the age interval 50 to 100,  $\ln(S_{i,100}/S_{i,50})$ . Using the life table for Swedish females born in 1898–1902, the average relative risk between ages 50 and 100 was 0.90 for  $\epsilon_{2/3}$  and 1.17 for  $\epsilon_{3/4}$ , which are very close to the final values given in Table III. However, between ages 50 and 90, the average relative risks were quite different: 0.86 and 1.26.

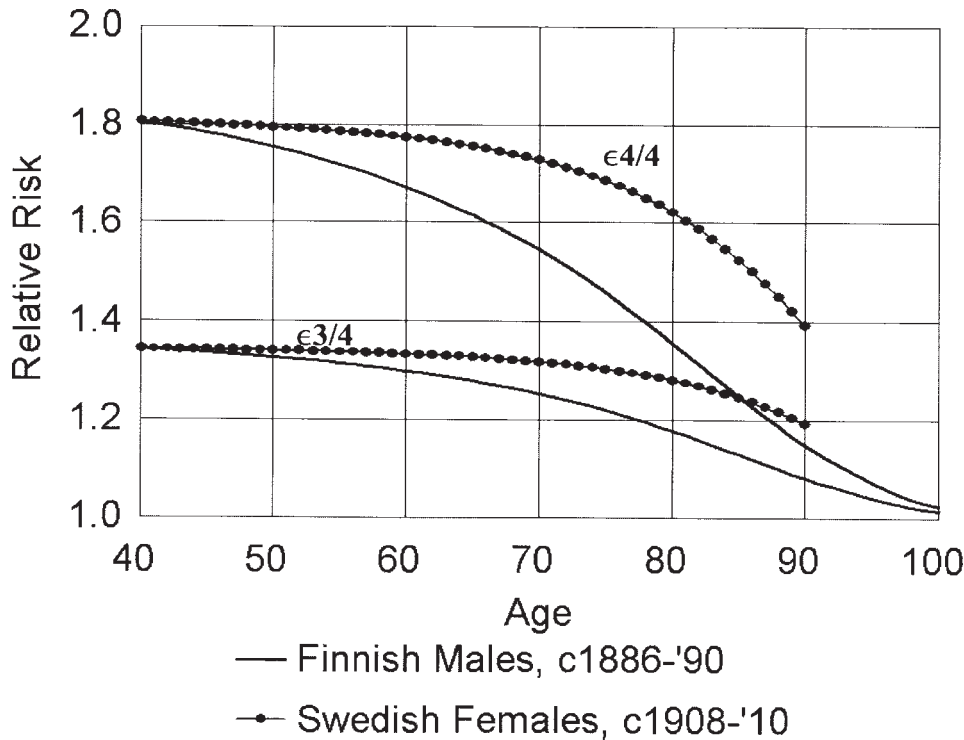


Fig. 1. Estimated risks of death for APOE.

### Test of the Appropriateness of the Gamma Distribution

The estimate of  $l$ , 1.13, is close to 1.0 and significantly different from 2.0 ( $P = 0.03$ ). Therefore, changes in the relative risks with age are more consistent with the Gamma than the Inverse Gaussian distribution. Using only the data from Finland, the country with the most data, led to a similar estimate of  $l$ , 1.18.

### Test of Differences in the Effects of APOE Across Populations

Chi square tests applied to the data from each study found no significant differences between any of the studies and the estimates from the model. Likelihood ratio tests were used to compare the data from different regions. Among females and sexes combined, there was no significant difference between relative risks estimates for Northern Europe (Finland, Sweden and Denmark) and Southern Europe (France and Italy). Among males there was a significant regional difference in the estimated relative risk for  $\epsilon 3/4$  allele (1.49 in Northern Europe and 1.12 in Southern Europe,  $P = 0.01$ ). However, this comparison relied completely on the data from French centenarians. There were no significant differences in the effect of APOE on mortality within Scandinavia.

### Evaluation of the Need for Cohort Life Tables in the Estimation

The parameters were re-estimated using a single, recent life table: the U.S. life table for 1989–1991. This led to a lower relative risk for the  $\epsilon 2/3$ , 0.80, and a higher relative risk for the  $\epsilon 3/4$ , 1.42. Using only the life table for Swedes born in 1898–1902 led to estimates very close to the final estimates given in Table III. However, using the life table for Finns born in 1886–1890 led to a relative risk for  $\epsilon 3/4$  of 1.25, which is substantially below the final estimate.

## DISCUSSION

Demographic synthesis offers an alternative to logistic regressions and meta-analysis. It uses a demographic model to combine estimates of different quantities into estimates of a unified set of parameters. Like the related methods proposed by Yashin et al. [1999], it uses life table data to model changes in gene frequencies by age. The model adds the use of data from cohort studies and a test for the appropriate assumption about the distribution of frailty. The use of cohort life tables adjusts for the fact that the gene frequencies decline with age faster in populations with higher mortality rates.

The APOE  $\epsilon 4$  allele is associated with an elevated risk of death in populations of European origin (relative risk at age 40 of 1.34, 95% CI 1.18–1.67). The  $\epsilon 4/4$  genotype can be assumed to be the square of the effect of the  $\epsilon 3/4$  genotype at the younger ages, 1.81. The  $\epsilon 2$  allele is associated with a significant reduction in mortality (0.84, 95% CI 0.68–0.93). APOE genotypes are associated with little variation in mortality at age 100. The results demonstrate that the risks diminish with age, not that these changes are caused by fixed frailty. However, the close match of the age pattern of decline in the relative risks with those that would be expected from the Gamma distribution of frailty is impressive.

There is no evidence that the relative risks for APOE differ geographically within Europe for females. There is some evidence from data on French centenarians that the relative risk for  $\epsilon 3/4$  in males is higher in Scandinavia than in southern Europe. The use of cohort life tables makes a noticeable difference in the estimates of the relative risks. This is contrary to the assumption made by previous researchers [Toupance et al., 1998; Yashin et al., 2000].

Demographic synthesis provides a way of comparing and combining data from a wide

range of study designs with diverse sample compositions. Therefore, it is useful for summarizing numerous, often conflicting, results from genetic studies that may not be directly comparable.

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## APPENDIX

### Life Tables for Cohorts

Database, BMD [Wilmoth, 2000], The main sources for sex-specific cohort life tables were the Berkeley Mortality various volumes of the United Nations Demographic Yearbook, UNDY, and Kannisto [1996]

*Denmark.* For the cohort born in 1890–1894 the UNDY was used for ages 50–84 and Kannisto for later ages. Under age 50, BMD data for Swedes born in 1898–1902 were scaled to match the level of mortality in Denmark. The UNDY was used for the cohort born in 1948–1952.

*Italy.* Data for the cohort born 1935–1940 and for the cohort of 1892–1897 for ages 45–84 were from the UNDY. Under age 45, rates for the French cohort of 1895 from the BMD were adjusted to the level of mortality in Italy. Rates after age 85 were based on Kannisto.

*Finland.* Data for cohorts born 1886–1890, 1891–1895, 1901–1905, 1911–1915, and 1948–1952 up to 1970 are from Kolari [1980]. Data for 1970–1995 up to age 85 come from UNDY. Rates over age 85 were based on Kannisto [1996] adjusted for a 1% change in mortality per year.

*France.* Life tables for the cohorts born in 1895 and 1945 were based on BMD.

*Sweden.* Data for Swedes born 1898–1902, 1908–1910, and 1958–1962 were from BMD.

*U.S. Females.* Life tables for women born in 1898–1902, 1903–1907, 1908–1912, and 1918–1922 were derived from BMD.