Effect of the Interplay Between Genetic and Behavioral Risks on Survival After Age 75

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OBJECTIVES: To explore the association between genes that may be related to human mortality, taking into account the possible contribution of morbidity, and investigate whether lifestyle behaviors may attenuate genetic risk.

DESIGN: Twenty-five-year population-based cohort study.

SETTING: Kungsholmen cohort, Stockholm, Sweden.

PARTICIPANTS: Individuals aged 75 and older (N = 1,229).

MEASUREMENTS: The associations between single-nucleotide variations in 14 genes (previously associated with mortality or to diseases linked to mortality), relevant lifestyle risk behaviors (smoking; mental, physical, or social inactivity; moderate or poor social network), and mortality were estimated using Cox regression.

RESULTS: People with allelic variation in four genes related to cardiovascular diseases and metabolism were more likely to die: apolipoprotein *(APO)C1* GG and AG carriers, *APOE* ε4 carriers, insulin-degrading enzyme (*IDE*) TC carriers, and phosphatidylinositol 3-kinase (*PI3KCB*) GG carriers. Individuals with multiple adverse alleles had 62% higher mortality rate than those with none. In contrast, people with no risk behaviors (low-risk profile) had 65% lower mortality rate than people with all examined risk behaviors (high-risk profile). Combining the genetic and environmental factors, it was found that, independent of genetic profile, individuals with a low-risk profile had up to 64% lower mortality rate than those with a moderate high– or high-risk profile and at least one genetic risk factor.

CONCLUSION: This study supports and expands evidence that genetic variations in *APOE*, *IDE*, and *PI3KCB* are associated with lower mortality rate, although lifestyle behaviors can modulate their effects. **J Am Geriatr Soc 2016.**

Key words: genes; lifestyle behaviors; survival; mortality; population-based cohort study

Longevity is a multifactorial quantitative trait that genetic, environmental, biomedical, and stochastic factors affect.[1] Several studies have shown that lifestyle behaviors and related factors such as smoking, alcohol consumption, and body weight can predict mortality in elderly people.[2–6] Social networks[7] and leisure-time activity, especially physical activity,[2] are also associated with survival in elderly adults. In a previous study of adults aged 75 and older, a group with the lowest-risk profile, characterized by healthy lifestyle behaviors (never smoking, normal weight), participation in at least one leisure activity, and a rich social network, was identified. These people lived 5 years longer than persons with all risk behaviors.[8] That study did not take into account participants' genetic background. Although the genetic contribution to longevity appears to be minimal before age 65, its influence increases with age after age 85.[9] Of the many genes that it has been proposed may be relevant to longevity, only apolipoprotein *(APO)E* is consistently found to be associated with longer survival.[10,11] Few human studies have examined the effect of the interplay between genes and lifestyle behaviors on longevity.[12,13]

Research suggests that there are multiple ways to achieve exceptional longevity and that no single factor is necessary or sufficient to determine the aging phenotype at the individual level. In this study, the hypothesis that lifestyle behaviors may attenuate genetic risk of shorter survival was tested. Of potential candidate genes, 14 associated with diseases that have a clear effect on survival

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or with mechanisms that influence longevity were selected; behaviors already identified as powerful predictors of longer survival were examined.[8]

METHODS

Study Population

Data were used from the Kungsholmen Project, a community-based longitudinal study on aging and dementia.[14] The cohort included all registered inhabitants in the Kungsholmen district of central Stockholm, Sweden, aged 75 and older in October 1987. After baseline examination, five follow-up examinations were completed at 3-year intervals before direct examination was terminated in 2000. Information from death certificates was collected up to 2013. Of the 1,810 participants examined at baseline, 581 did not provide blood for deoxyribonucleic acid (DNA) preparation, leaving 1,229 participants in the current study sample.

The ethics committee of Karolinska Institutet approved all parts of the project, and participants provided written informed consent.

Data Collection

Data on age, sex, and education were obtained from participants in face-to-face interviews with trained nurses following standardized protocols.[14] Educational level was measured as total years of formal schooling and divided into primary (≤7 years) and secondary school or above (≥8 years).

Information on smoking was obtained from the baseline interview or, if information was missing, from data collected at the first follow-up, 3 years later. Smoking history was assessed by asking whether the participant had ever smoked. Former smokers were asked at what age they had stopped smoking, and smoking status was categorized as current, former, or never. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and was analyzed as a categorical variable: low (<20 kg/m^2), normal (20–25 kg/m^2), or high (>25 kg/m^2).

Information on leisure activities and social networks was obtained from the baseline interview.[14] Participants were asked whether they regularly engaged in any leisure activities. If so, they were asked to specify the types of activities and to report the frequency of participation. Two researchers independently assigned a physical, mental, and social activity component score to each activity on the basis of the nature of the type of activity. If consensus could not be reached, they then discussed the component score with a third researcher. The grading of the three components was coded as 0 (none), 1 (low), 2 (moderate), or 3 (high).[15] Owing to the statistical power of the study, activity was dichotomized as any participation versus no participation. To determine the extent of social networks, participants were asked about marital status, living arrangements, parenthood, and friendships. Frequency of contact with children and friends or relatives and how satisfied participants were with the frequency of those contacts were asked about. On the basis of their answer, participants were grouped into the three social network categories of rich, moderate, and poor.[16]

Based on a previous publication,[8] a risk profile was created considering the following modifiable lifestyle behaviors: smoking status, participation in leisure activities (physical, mental, social), and social network. The following four risk profiles were defined: high (current smokers, no leisure activities, moderate or poor social network), moderately high (two of the three risk factors), moderately low (one risk factor), and low (none of the three risk factors).

On the basis of clinical examination, medical history, laboratory data, and current drug use, the examining physician diagnosed all chronic diseases for each person. Participants' history of diseases was also ascertained using the Swedish National Inpatient Register. This register includes records from 1969 onward; data were used from 1969 to 2000 for the current study. Diagnoses were based on the *International Classification of Diseases*, Eight, Ninth, and Tenth Revisions (ICD-8, 9, 10). Based on the literature, the diseases that were selected were those associated with the studied genes, such as dementia, diabetes mellitus, ischemic heart disease (IHD), and cerebrovascular disease. A three-step procedure was used to define dementia cases; two physicians independently made preliminary diagnosis, and if there was disagreement, a third opinion was obtained. The *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised*, criteria were used in the diagnosis of dementia and dementia type. Dementia types include Alzheimer's disease, vascular dementia, mixed dementia, alcoholic dementia, dementia in Parkinson's disease, and unspecified dementia. Diabetes mellitus was considered to be present if the participant had a diabetes mellitus diagnosis (ICD-8 and 9 code 250; ICD-10 code E11), if the participant was taking antidiabetic drugs (hypoglycemic medications or insulin injection, ATC code A10), or if his or her blood glucose level was higher than 11 mmol/L. IHD and cerebrovascular disease were diagnosed according to the ICD (ICD-8 and 9 code 410–414, ICD-10 code I22–25 for IHD; ICD-8 and 9 code 430–438, ICD-10 code I60–69 for cerebrovascular disease). If participants were not able to answer the questions (e.g., because of cognitive impairment), an informant, usually a close relative, was interviewed.

Genotyping Procedure

Genomic DNA was extracted from peripheral blood samples. Genotyping was performed using MALDI-TOF analysis on the Sequenom MassARRAY platform at Karolinska University Hospital, Huddinge. The genotyping procedure used has been described in detail elsewhere.[17] Allelic variations in several genes that have previously been found to be associated with mortality or to diseases linked to mortality were investigated: angiotensin-converting enzyme (*ACE*; rs4343, rs1800764), *APOB* (rs693), *APOC1* (rs4420638), *APOE* (rs429358), brain-derived neurotrophic factor (rs6265), fat mass and obesity-associated protein (rs9939609), hydroxy-methyl-glutaryl-CoA reductase (rs3761740), insulin-degrading enzyme (*IDE*; rs1887922, rs1544210), insulin-like growth factor 1 receptor (*IGF1R*; rs2229765), interleukin 6 (rs1800795), lipase

(rs1800588), lipoprotein lipase (rs328), methylene tetrahydrofolate reductase (rs1801133), and phosphatidylinositol 3-kinase (*PIK3CB*; rs361072).

Statistical Analysis

Hardy-Weinberg equilibrium was tested for all genotypes, and no deviation was detected. Linkage disequilibrium between single nucleotide polymorphisms (SNPs) was estimated using standardized disequilibrium coefficients (D') and squared allele-frequencies correlation (r^2).[18] The association between the SNPs under investigation and death was evaluated in terms of mortality rate, with the reference category being the homozygote of the major allele. Cox regression models were used to estimate hazard ratios (HRs) of mortality using age as a time-scale. Analyses were corrected for delayed entry so that individuals were considered at risk from age at entry into the cohort.[19] The proportional hazards assumption was assessed by regressing the scaled Schoenfeld residuals against survival time. No departure from the assumption was detected. Survival time was censored for those who were still alive at the end of the study (February 8, 2013). A sensitivity analysis was performed by restricting follow-up to 10 years.

The association between variations in the selected genes and mortality was first assessed, controlling for sex differences. Approximately 32% of the population did not provide blood for DNA. Age and mental inactivity were related to greater odds of missing genotype data, whereas being diagnosed with dementia was related to lower odds of missing genotype data after accounting for sex, education, smoking status, BMI, leisure activities, and social network. The proportion of missing covariate data was 1.0% for education, 18.8% for BMI, and 28% for smoking. A complete case analysis was conducted based on 68% of the cohort. A sensitivity analysis was performed for missing data, with multiple imputations by chained equations to obtain 50 imputed datasets. The estimates were pooled using Rubin's rule to obtain valid statistical inferences.[20] All relevant variables and the outcome were used in the imputation models. The magnitude and direction of the estimates based on complete case and multiple imputation were overall similar, so it was decided to present the results from the multiple imputation.

Then, to estimate the relative contribution of chronic conditions (e.g., dementia, IHD, cerebrovascular disease, diabetes mellitus) to the genotype-mediated mortality association, models where constructed including the chronic condition. The chronic conditions were considered as time-dependent variables. On the basis of the previous analysis, a genetic risk score was created by counting the numbers of adverse alleles, taking into account the possible correlation between genes. The association between having multiple adverse alleles and mortality was determined, accounting for age, sex, lifestyle risk behaviors, and chronic conditions. Finally, the association between mortality and the combination of genes and lifestyle risk behaviors was evaluated. A dummy variable was created based on every possible combination of the two variables: people with at least one adverse allele and with moderately high– or high-risk profile as reference group.

Statistical interactions between genes and lifestyle risk behaviors in predicting mortality were evaluated. Statistical interactions do not necessarily imply the presence of causal interactions,[21] so the presence of additive interaction between variation in each single genes and the lifestyle risk profile was also tested using a previously proposed formula[22] as a measure of relative excess risk due to interaction (RERI).

Analyses were performed using Stata, version 14 (Stata Corp LP, College Station, TX).

RESULTS

During the 25-year follow-up period, 1,218 (99.1%) deaths occurred. Table 1 shows baseline characteristics of study participants (n = 1,229) stratified according to sex. Mean age ± SD at baseline was 80.6 ± 4.5 for men and 81.5 ± 5.0 for women. Men were more likely than women to be highly educated and to be current smokers, to participate in mental activity, and have a more-developed social network and less likely to have dementia (Table 1).

Table 2 shows the sex-adjusted HRs of mortality according to the variation in each gene. Of all the candidate genes, a significant association was found between variations in *APOC1*, *APOE*, *IDE* (rs1544210), and *PIK3CB* and mortality. Twenty-two percent higher mortality rate was found in *APOC1* AG carriers than AA carriers. The 45 participants with the *APOC1* GG genotype had 60% higher mortality than the AA carriers; because of the small sample size, mortality associated with having any *APOC1* G allele was also estimated (complete case analysis: HR = 1.25, 95% confidence interval (CI) = 1.11–1.41; multiple imputation analysis: HR = 1.25, 95% CI = 1.10–1.41). People with the *APOE* ε4 allele had 24% higher

Table 1. Baseline Characteristics of Study Population According to Sex (N = 1,229)

Characteristic	Male, n = 311	Female, n = 918
Age, mean ± standard deviation	80.6 ± 4.5	81.5 ± 5.0
Education secondary school or greater, n (%)	176 (56.6)	404 (44.4)
Body mass index high or low, n (%)	124 (43.7)	386 (49.1)
Lifestyle risk behavior, n (%)		
Current smoker	48 (20.7)	70 (9.5)
Physical inactivity	273 (90.7)	801 (89.9)
No social activity	140 (45.0)	466 (50.8)
No mental stimulation	96 (30.9)	341 (37.1)
Moderate or poor social network	265 (85.2)	880 (95.9)
Dementia, n (%)		
Prevalent	22 (7.1)	89 (9.7)
Incident	65 (20.9)	293 (31.9)
Ischemic heart disease, n (%)		
Prevalent	36 (11.6)	81 (8.8)
Incident	77 (24.8)	211 (23.0)
Cerebrovascular disease, n (%)		
Prevalent	28 (9.0)	55 (6.0)
Incident	70 (22.5)	211 (23.0)
Diabetes mellitus, n (%)		
Prevalent	13 (4.2)	22 (2.4)
Incident	15 (4.8)	52 (5.7)
Alive after 25 years, n (%)	0 (0.0)	11 (1.2)

Table 2. Sex-Adjusted Hazard Ratio of Mortality According to Single-Nucleotide Polymorphism (SNP)

SNP	Total/Deaths, n/n	Hazard Ratio of Mortality (95% Confidence Interval)	
		Complete Case	Multiple Imputation
ACE (rs4343)			
GG	311/309	Reference	Reference
AG	600/593	0.96 (0.84–1.10)	0.97 (0.84–1.11)
AA	286/284	1.04 (0.89–1.22)	1.05 (0.89–1.22)
ACE (rs1800764)			
TT	371/367	Reference	Reference
TC	594/590	0.97 (0.85–1.11)	0.97 (0.85–1.10)
CC	250/247	0.93 (0.79–1.10)	0.92 (0.79–1.07)
APOB (rs693)			
AA	339/337	Reference	Reference
AG	589/584	0.95 (0.83–1.09)	0.97 (0.86–1.10)
GG	280/276	0.85 (0.73–1.00)	0.89 (0.76–1.04)
APOC1 (rs4420638)			
AA	779/771	Reference	Reference
AG	379/376	1.22 (1.07–1.38)	1.20 (1.07–1.36)
GG	45/45	1.60 (1.18–2.16)	1.62 (1.24–2.12)
APOE (rs429358)			
$\varepsilon3\varepsilon3$	660/652	Reference	Reference
$\varepsilon2\varepsilon3$	161/159	0.83 (0.70–0.99)	0.89 (0.77–1.04)
Any $\varepsilon4$	355/354	1.24 (1.10–1.42)	1.24 (1.09–1.40)
Brain-derived neurotrophic factor (rs6265)			
CC	831/822	Reference	Reference
CT	344/343	0.97 (0.85–1.10)	0.96 (0.85–1.09)
TT	39/38	0.81 (0.58–1.12)	0.83 (0.60–1.14)
Fat mass and obesity-associated gene (rs9939609)			
TT	397/394	Reference	Reference
AT	566/560	1.07 (0.94–1.21)	1.07 (0.95–1.21)
AA	212/210	1.08 (0.91–1.28)	1.08 (0.91–1.27)
Hydroxy-methyl-glutaryl-CoA reductase (rs3761740)			
CC	983/975	Reference	Reference
AC	213/210	0.89 (0.76–1.03)	0.91 (0.78–1.06)
AA	17/17	1.09 (0.67–1.78)	1.14 (0.77–1.69)
IDE (rs1887922)			
TT	854/845	Reference	Reference
TC	340/338	1.20 (1.06–1.36)	1.17 (1.03–1.31)
CC	30/30	0.97 (0.67–1.39)	0.91 (0.59–1.40)
IDE (rs1544210)			
AA	331/327	Reference	Reference
AG	575/570	0.97 (0.84–1.11)	1.00 (0.88–1.15)
GG	315/313	0.93 (0.80–1.09)	0.95 (0.83–1.09)
Insulin-like growth factor-1R (rs2229765)			
GG	337/335	Reference	Reference
AG	617/613	0.99 (0.87–1.14)	1.01 (0.89–1.16)
AA	253/248	0.96 (0.81–1.13)	0.98 (0.83–1.14)
Interleukin-6 (rs1800795)			
GG	339/335	Reference	Reference
CG	611/609	1.04 (0.90–1.21)	1.03 (0.91–1.16)
CC	262/257	1.00 (0.85–1.18)	1.02 (0.86–1.19)
Lipase (rs1800588)			
CC	727/719	Reference	Reference
CT	520/418	0.93 (0.82–1.06)	0.96 (0.85–1.08)
TT	55/54	0.78 (0.59–1.03)	0.78 (0.57–1.08)
Lipoprotein lipase (rs328)			
CC	1,018/1,009	Reference	Reference
CG	196/194	0.92 (0.79–1.07)	0.96 (0.83–1.11)
GG	8/8	0.51 (0.25–1.02)	0.64 (0.37–1.11)
Methylene tetrahydrofolate reductase (rs1801133)			
GG	600/496	Reference	Reference
AG	465/460	0.97 (0.86–1.09)	0.99 (0.89–1.10)
AA	116/114	1.02 (0.83–1.24)	0.97 (0.81–1.16)
PIK3CB (rs361072)			
AA	387/382	Reference	Reference
AG	606/601	1.10 (0.96–1.25)	1.06 (0.95–1.21)
GG	220/219	1.20 (1.01–1.41)	1.16 (1.01–1.36)

ACE = angiotensin-converting enzyme; APO = apolipoprotein; IDE = insulin-degrading enzyme; PIK3CB = phosphatidylinositol 3-kinase.

mortality than $\varepsilon3\varepsilon3$ carriers. Twenty percent higher mortality was found in *IDE* (rs1887922) TC than TT carriers and in *PIK3CB* GG than AA carriers. There was no difference in mortality for all the other genotypes examined (Table 2). As discussed in the Methods, the magnitude and direction of the estimates based on complete case and multiple imputations were overall similar (Table 2).

APOC1 and *APOE* genotypes affect the risk of dementia, IHD, and cerebrovascular disease. Therefore, to verify whether those diseases may partially or completely explain the association between *APOC1* and *APOE* and survival, the association between variations in those genes and survival was estimated after adjusting for IHD, cerebrovascular disease, and dementia. As expected, mortality in *APOC1* AG and *APOE* $\varepsilon4$ carriers was significantly lower after adjustment for dementia but not after adjustment in participants with IHD and cerebrovascular disease (Table 3). Moreover, linkage disequilibrium with the *APOE* allele $\varepsilon4$ fully explains the association between the *APOC1* AG genotype and mortality ($D' = 0.90$, $r^2 = .64$, $P < .001$). *IDE* and *PIK3CB* have been found to be associated with higher risk of diabetes mellitus. To verify whether diabetes mellitus may explain these associations, it was included in the model. The higher mortality associated with the *IDE* TC genotype was unaffected after adjustment for diabetes mellitus, whereas the higher mortality associated with the *PIK3CB* GG genotype was no longer significant after adjustment for diabetes mellitus (Table 3).

Once all of the selected genes and the diseases (dementia, IHD, cerebrovascular diseases, and diabetes) were included in the same model, *APOE* $\varepsilon4$ carriers had 17% higher mortality (HR = 1.17, 95% CI = 1.03–1.33), *IDE* TC carriers had 20% higher mortality (HR = 1.20, 95% CI = 1.05–1.38), and *PIK3CB* GG carriers had 18% higher mortality (HR = 1.18, 95% CI = 1.01–1.39). It was decided not to include the *APOC1* gene in further analysis because of linkage disequilibrium detected with *APOE*. When the *APOC1* gene was included instead of *APOE*, similar results were found (HR = 1.16, 95% CI = 1.03–1.31 for any *APOC1* G allele; HR = 1.21, 95% CI = 1.05–1.38 for *IDE* TC genotype; HR = 1.19, 95% CI = 1.01–1.39 for *PIK3CB* GG genotype).

Next, multivariable-adjusted associations between number of adverse alleles, lifestyle risk profile, and mortality were examined, taking into account personal

Table 3. Hazard Ratio of Mortality After 25 Years of Follow-Up Using Cox Proportional Hazards Models

Single-Nucleotide Polymorphism	Adjusted for Sex	Adjusted for Sex, Ischemic Heart Disease, Cerebrovascular Disease	Adjusted for Sex, Dementia	Adjusted for Sex, Diabetes Mellitus, Body Mass Index
	Hazard Ratio of Mortality (95% Confidence Interval)			
*APOC*1 (rs4420638) (reference AA)				
AG	1.20 (1.07–1.36)	1.21 (1.07–1.37)	1.12 (1.00–1.26)	
GG	1.62 (1.24–2.12)	1.44 (1.09–1.93)	1.37 (1.04–1.81)	
APOE (rs429358) (reference ε3ε3)				
ε2ε3	0.89 (0.76–1.04)	0.94 (0.81–1.10)	0.91 (0.77–1.07)	
Any ε4	1.24 (1.09–1.40)	1.24 (1.10–1.41)	1.12 (0.99–1.27)	
Insulin-degrading enzyme (rs1887922) (reference TT)				
TC	1.17 (1.03–1.31)			1.17 (1.04–1.32)
CC	0.91 (0.59–1.40)			0.91 (0.59–1.40)
Phosphatidylinositol 3-kinase (rs361072) (reference AA)				
AG	1.06 (0.92–1.22)			1.07 (0.93–1.23)
GG	1.16 (1.01–1.36)			1.16 (0.99–1.36)

Estimates were derived from multiple imputation analysis.
APO = apolipoprotein.

characteristics and chronic conditions. The results are expressed as HRs of mortality and 95% CIs in Table 4. As expected, women had lower mortality than men (HR = 0.64, 95% CI = 0.57–0.72). Having one, two, or three adverse alleles (*APOE*, *IDE*, *PI3KCB*) was progressively associated with higher mortality (28%, 30%, 62%) (*P*-value per trend <.01). In contrast, people with a low-risk profile (never smokers, engaged in at least one leisure activity, rich social network) had 65% lower mortality than those with a high-risk profile (current smokers, not engaged in any leisure activity, and with a moderate or poor social network). Mortality of people with dementia, IHD, or diabetes mellitus was almost 50% higher and was twice as high for those with cerebrovascular disease as for those without (Table 4).

There were no indications of multiplicative interaction between genes and lifestyle behaviors or lifestyle risk profile (*P*-values ranged from .08 to .98). Moreover, no evidence was found of additive interaction between the *APOE* ε4 allele and having a moderately low– (RERI = 0.32, 95% CI = −0.41 to 1.06, *P* = .39) and moderately high– or high-risk profile (RERI = 0.10, 95% CI = −0.68 to 0.88, *P* = .80) or between the *APOC* G allele and having a moderately low– (RERI = 0.13, 95% CI = −0.63 to 0.88, *P* = .74) and moderately high– or high-risk profile (RERI = 0.18, 95% CI = −0.61 to 0.97, *P* = .65). No significant additive interaction was found between *IDE* C allele and having a moderately low– (RERI = 0.09, 95% CI = −0.92 to 1.09, *P* = .87) or moderately high– or high-risk profile (RERI = 0.12, 95% CI = −0.90 to 1.14, *P* = .82) or between *PI3KCB* G allele and having a moderate low– (RERI = 0.22, 95% CI = −0.43 to 0.87, *P* = .50) or moderately high– or high-risk profile (RERI = 0.09, 95% CI = −0.63 to 0.82, *P* = .80).

Figure 1 shows the association between mortality, genetic profile, and lifestyle risk profile. Despite the presence of the adverse alleles, individuals with a low-risk profile (no smoker, engaged in at least one leisure activity, rich social network) had up to 64% lower mortality than individuals with a moderately high– or high-risk profile (two or more lifestyle risk behaviors (current smoker, moderate or poor social network, no engagement in leisure activities), one or more adverse alleles) (Figure 1).

An additional analysis was conducted in which survival time was restricted to 10 years of follow-up. In agreement with the results estimated using the full follow-up, higher mortality was detected in people with allelic variation in *APOC1* (HR = 1.21, 95% CI = 1.07–1.37 for AG vs AA carriers), *APOE* (HR = 1.22, 95% CI = 1.07–1.40 for any ε4 vs ε3ε3 carriers), *IDE* (HR = 1.13, 95% CI = 1.01–1.26 for TC vs TT carriers), and *PI3KCB* (HR = 1.15, 95% CI = 1.01–1.35 for GG vs AA carriers). Combining the genetic and environmental factors, it was found that, independent of genetic profile, individuals with a low-risk profile had up to 70% (HR = 0.30, 95% CI = 0.17–0.53) lower mortality than individuals with a higher-risk profile and at least one genetic risk factor.

DISCUSSION

The effect of the interplay between genes and lifestyle behaviors on survival was investigated taking into account genes that have been more frequently associated with longer survival and risk behaviors identified in a previous study derived from the same cohort. Variations in four genes (*APOC1*, *APOE*, *IDE*, *PI3KCB*) were associated with higher mortality and having multiple adverse alleles increased mortality by up to 62%, although a behavioral low-risk profile (no smoking; physical, mental, social activity; moderate or rich social network) attenuated the higher mortality associated with genetic susceptibility. Regardless of their genetic status, participants with a low-risk profile had approximately 64% lower mortality than those with a high-risk profile (two or more of the risk behaviors).

The higher mortality found in this study in people with the *APOE* ε4 allele confirms previous longitudinal findings.[10,11,23–25] Various hypotheses have been proposed surrounding the link between *APOE* and mortality, but

Table 4. Hazard Ratio of Mortality After 25 Years of Follow-Up According to Cox Proportional Hazards Model

Variable	Multivariable Adjusted Hazard Ratio (95% Confidence Interval)
Personal characteristics	
Female	0.64 (0.57–0.72)
Education secondary or above	0.91 (0.83–1.01)
High or low body mass index	1.14 (1.02–1.27)
Number of adverse alleles[a]	
1	1.28 (1.13–1.44)
2	1.30 (1.11–1.52)
3	1.62 (1.05–2.50)
Lifestyle risk profile (reference high[b])	
Moderately high[c]	0.75 (0.59–0.96)
Moderately low[d]	0.53 (0.42–0.66)
Low[e]	0.35 (0.24–0.51)
Chronic conditions[f]	
Dementia	1.51 (1.36–1.67)
Ischemic heart disease	1.50 (1.35–1.67)
Cerebrovascular diseases	2.13 (1.92–2.36)
Diabetes mellitus	1.47 (1.25–1.74)

Estimates were derived from multiple imputation analysis.
[a]Considering the following genes: apolipoprotein E, insulin-degrading enzyme (rs1887922), and phosphatidylinositol 3-kinases.
[b]Smokers, no participation in leisure activity, moderate or poor social network.
[c]Two of the lifestyle risk behaviors.
[d]One lifestyle risk behaviors.
[e]No risk behaviors.
[f]Prevalent and incident cases.

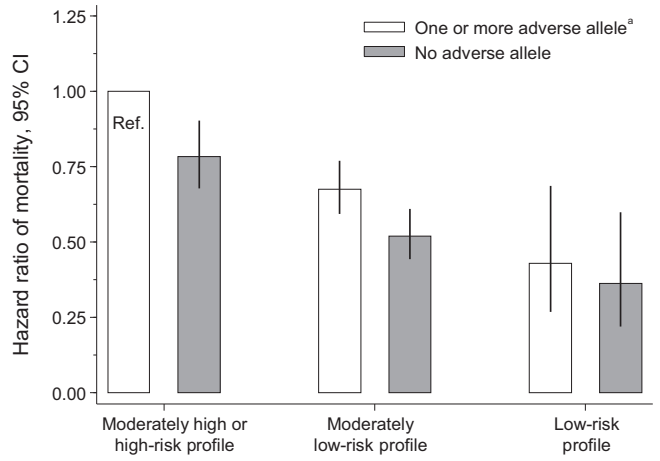

Figure 1. Association between mortality and the combination of genetic and behaviors risks. The values are expressed as hazard ratios of mortality with 95% confidence intervals adjusted for age, sex, education, body mass index, dementia, ischemic heart disease, cerebrovascular disease, and diabetes mellitus. Estimates were derived from multiple imputation analysis. High-risk profile: smoker, no participation in leisure activities, moderate or poor social network. Moderately high–risk profile: two of the lifestyle risk behaviors. Moderately low–risk profile: one lifestyle risk behaviors, Low-risk profile: no risk behaviors. [a]Considering the following genes: apolipoprotein E, insulin-degrading enzyme (rs1887922), and phosphatidylinositol 3-kinase.

the exact mechanisms underlying the association between *APOE* and mortality have yet to be determined. *APOE* has roles beyond lipoprotein metabolism. The *APOE* isoforms generated from the various alleles interact differently with the lipoprotein receptors, leading to different cholesterol levels. High levels of low-density lipoprotein cholesterol are associated with *APOE* ε4. High levels of low-density lipoprotein cholesterol lead to atherosclerosis, increasing the risk of heart attack and ischemic stroke.[26] The *APOE* ε4 allele is also associated with greater risk of Alzheimer's disease.[10,11] The second association detected was with *APOC1*; G carriers had higher mortality than AA carriers. No other previous studies have examined this SNP in relation to mortality, but *APOC1* is located on chromosome 19 in the same region as the *APOE* gene, and these findings seem to reflect linkage disequilibrium between the *APOC1* and *APOE* genes. The majority of the effect of the *APOE* ε4 allele and *APOC1* AG genotype on mortality could be attributed to dementia in the current study, whereas IHD and cerebrovascular disease did not modify the effect. The other genes associated with higher mortality in the current study were the *IDE* and *PI3KCB* genes. In a variety of animal models from invertebrates to mammals, data indicate that the insulin/IGF-1 signal pathway regulates aging.[27] In humans, insulin sensitivity normally decreases with age, and insulin resistance is an important risk factor for metabolic syndrome,[28] which ultimately affects mortality in elderly people.[29–31] Previous research has shown that variation in the *IGF-1* receptor and *PIK3CB* gene can affect IGF-1 plasma levels and might affect longevity.[30] Moreover, the *IDE* gene is one of the central regulators of insulin metabolism and participates in intercellular peptide signaling by degrading a variety of proteins such as IGF-2 and β-amyloid.[32] *IDE* has also been associated with several diseases: Alzheimer's disease[33] and type II diabetes mellitus.[34] These observations prompted us to investigate whether genes that encode components of this pathway are likely to be involved in survival. Previous research has shown an association between genetic variations in *IDE*, *IGF-1R*, and *PIK3CB* and longevity.[29–31,35] The effect of the *IDE* and *PIK3CB* genes was confirmed; the analyses showed approximately 20% higher mortality in people with the TC genotype of the *IDE* gene and the GG genotype of the *PIK3CB* gene. Body composition and diabetes mellitus explained the effect of *PIK3CB* but not of *IDE*. No significant association was found between the *IDE* CC genotype and mortality. This lack of association might reflect the small sample size of *IDE* CC carriers (n = 30), which may have led to false-negative results. This result must therefore be interpreted with caution.

There has been limited evidence of the possible interaction between genes and lifestyles on survival to advanced ages. The current study examined the possibility of such an interaction and found that the mortality associated with genetic susceptibility was attenuated by 64% in people with healthy lifestyle behaviors. In agreement with these findings, a cohort study of people aged 39 to 79 found that physical activity reduced genetic risk to obesity by 40%.[12] Another study that assessed the contribution of genetic factors to mortality in people aged 55 and older also reported that lifestyle factors attenuated genetic risk.[13]

In line with these results, in elderly people, high education; a socially, mentally, and physically active life; and a low vascular burden reduced the risk of dementia related to *APOE* ε4.[36] Because dementia is strongly related to mortality,[37] the attenuation of genetic risk by environmental factors found in dementia may at least partially explain the results of the current study.

Strengths of the present study include the long follow-up period, which allowed participants to be followed for up to 25 years, the extensive data on lifestyle behaviors, and the genetic data. Nevertheless, some limitations should be noted. Genetic association studies on the relationship between a complex phenotype (such as longevity) and susceptible genetic variants have shown that the majority of initial positive associations cannot be reproduced.[38] This suggests that some original findings are false positives or that small genetic effects were undetectable (false negatives). This might be because of small sample sizes, although it is possible that the lack of replicability may be due to true variability in the associations in different populations. Therefore, replication using larger samples is strongly recommended. The dropout rate at baseline in the Kungsholmen Project was 23.6% (12.4% declined to participate, 7.6% died, 3.6% moved out of the area), but the demographic characteristics of those who declined to participate and those who moved did not differ from those of the participants. Only the 181 persons who died differed from participants, being older and more often male. It is likely that the loss of these people led to an underestimation of the HRs, especially for the oldest men. Participants were better educated and had longer lifespans than the general population, which might affect the generalizability of the results. Finally, the population was followed from age 75 through 107, making the results relevant for old and very old individuals but not for younger persons.

In conclusion, this study extends past observations that genetic background is related to survival, but lifestyle behaviors attenuated the genetic risk, suggesting that that a long life can be achieved even in the presence of unfavorable genes. Given that similar behavioral risk factors are also related to major disorders in aging, such as cardiovascular disease and dementia,[39,40] it is likely that people with healthy lifestyles, in spite of an unfavorable genetic background, not only survive longer but are also healthier. These findings highlight the benefits of promoting and sustaining healthy lifestyles through the common efforts of individuals and healthcare systems.

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