From Aging Research Center (ARC),

Department of Neurobiology, Care Science and Society,
Karolinska Institutet, Stockholm, Sweden

Living longer than expected: protective and risk factors related to human longevity

Debora Rizzuto

Karolinska Institutet

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To the memory of my grandparents
nonna Gina , nonno Domenico
and
nonno Giovanni

ABSTRACT English

The scientific community has become increasingly interested in understanding what lies behind the continuing extension of the human lifespan. The main aim of this thesis was to better understand the association between health status, lifestyle, genetic factors and survival in advanced age. Data used in the 4 studies are gathered from the Kungsholmen Project, a longitudinal population-based study on 75 year and older participants living in Stockholm, Sweden.

Study I. Dementia, cardiovascular disease (CVD), and cancer were associated with a 2- to 3-fold increased rate of all-cause mortality. The mean survival times after incident diagnosis were 4.1 years for dementia, 4.2 years for CVD, and 2.2 years for cancer. A total of 3.4 potential years of life were lost because of dementia, 3.6 of CVD, and 4.4 because of cancer. Women aged 75 to 84 years lived longer than coetaneous men after incident diagnosis of dementia because they spent 1.6 years longer than men in the severe stage of the disease.

Study II. Findings suggest that *APOE* alleles play different roles in the survival of elderly women and men. The mortality rate was 40% lower among women, but not men, who carried the ε2 allele, compared with the ε3ε3 carriers. The ε4 allele was associated with a 50% higher rate of death only among men. Dementia, not ischemic heart and cerebrovascular diseases, accounted for the majority of the increased mortality rate in those with the ε4 allele.

Study III. Maintaining a healthy lifestyle and a rich social network was positively associated with survival even among people aged 75 years and older. People who reported being physically active a minimum of once a month lived about 2 years longer than those who did not. Non-smokers 75 years and older who participated in at least 1 leisure activity a month and had good social support lived about 5 years longer than inactive smokers with poor social support. These association, although attenuated, were also found in individuals aged 85 years and older and those with chronic diseases.

Study IV. Genetic risk factors were relevant for survival after age 75. Variations in 4 different genes (*APOC1*, *APOE*, *IDE*, and *PI3K*) were associated with 12–20% increased rate of mortality. However, participants with at least 1 risk allele and a healthy lifestyle had about 70% lower rate of death than those with no risk allele and an unhealthy lifestyle. Those with no risk alleles and a healthy lifestyle had 80% lower mortality rate and 6 years longer median lifespan than people with at least 1 risk allele and unhealthy lifestyle.

In **conclusion**, survival after 75 years of age was associated with health status, lifestyle, genetic factors, and a combination of those factors. These findings may help prognostic evaluation of the duration of specific diseases. They underscore the malignant nature of dementia as a result of the long period individuals lived with the severe disease stages, especially for women. These findings also suggest that the benefit of a healthy lifestyle, healthy behavior, and social support probably last a lifetime. Moreover, allelic variations in genes were associated with higher mortality rate, but the combined effect of genetic-environmental joint exposures may lead to the attenuation of the mortality rate, indicating that people with genetic susceptibility may reduce their initial mortality rate by modifying their lifestyle. Therefore, efforts to encourage smoking cessation, physical activity, and social engagement should be continued long into late life.

Key words: Mortality, age-related chronic diseases, lifestyle factors, genetic factors.

SAMMANFATTNING SVENSKA

Det finns ett stort intresse för att förstå de mekanismer som får människor att leva längre och längre. Syftet med denna avhandling är att öka förståelsen för sambanden mellan hälsa, livsstil, genetiska faktorer och överlevnad till hög ålder. Data till de 4 studierna kommer från Kungsholms projektet, en longitudinell studie av personer 75 år eller äldre, boende i Stockholm, Sverige.

Studie I. Demens, kardiovaskulära sjukdomar, and cancer var i den första studien associerade med en 2–3 faldig ökad risk för dödlighet. Antal år som personerna överlevde efter att de fått en diagnos var i medel 4,2 år för de med demens, 4,2 år för de med kardiovaskulär sjukdom och 4,2 år för de med cancer. Livet förkortades 3,4 år för personer med demens, 3,6 år för personer med kardiovaskulär sjukdom och 4,4 för personer med cancer. Kvinnor i åldern 75-84 levde längre efter att de fått en demensdiagnos än män i samma ålder, beroende på att de levde 1,6 år längre i ett senare och mera avancerat stadium av sjukdomen.

Studie II. Denna studie visar att variationer i *APOE* har olika betydelse för överlevnad hos kvinnor och män. Dödligheten var 40% lägre hos kvinnor, men ej hos män, som bar på *APOE* ε2. ε4 allelen var associerad med 50% högre risk för död enbart hos män. Demens var den faktor som förklarade majoriteten av den ökade risken för död hos ε4-bärare, medan kardiovaskulär sjukdom inte alls förklarade den ökade risken.

Studie III. Upprätthållandet av en hälsosam livsstil och ett rikt socialt nätverk hade ett positivt samband med överlevnad för personer som var 75 år och äldre. Personer som rapporterade att de var fysiskt aktiva åtminstone en gång per månad, levde ungefär 2 år längre än de som inte var fysiskt aktiva. Icke-rökare, 75 år och äldre, som deltog i åtminstone en fritidsaktivitet varje månad, och som hade ett bra socialt närverk, levde ungefär 5 år längre än rökare, som inte hade någon fritidsaktivitet och som hade ett dåligt socialt närverk. Detta kunde också observeras hos de som var 85 år och äldre, med kroniska sjukdomar, men effekten var försvagad hos denna grupp.

Studie IV. Genetiska riskfaktorer påverkade överlevnaden efter 75 års ålder. Variaitoner i 4 gener (*APOC1*, *APOE*, *IDE* och *PI3K*) visade 12–20% ökad risk för dödlighet. Trots det hade personer med minst en riskallel och en hälsosam och aktiv livsstil ungefär 70% lägre risk än de utan någon riskallel och en ohälsosam och inaktiv livsstil. Personer utan de genetiska riskfaktorerna och med en hälsosam och aktiv livsstil hade 80% lägre risk för att dö och medianvärdet för livslängden var 6 år längre än för de som bar på minst en riskallel och hade en ohälsosam och inaktiv livsstil.

Sammanfattningsvis är sannolikheten att leva längre än till 75 års ålder beroende av hälsostatus, livsstil, genetiska faktorer och en kombination av alla dessa faktorer. Fynden som presenteras i denna avhandling kan komma till nytta vid prognostiska utvärderingar av durationen specifika sjukdomar. De understryker demenssjukdomars elakartade karaktär, som följd av den långa tid människor lever i den allvarligaste och svåraste fasen av sjukdomen. Detta var speciellt uttalat för kvinnor. Dessa fynd pekar också på vikten av en hälsosam livsstil, ett hälsosamt beteende och ett fungerande socialt närverk för att leva länge. Det fanns ett samband mellan genetiska variationer och dödlighet, men resultaten indikerar att denna effekt kunde modifieras genom en hälsosammare livsstil. Slutsatsen är att ett hälsosamt leverne, så som att sluta röka, leva fysiskt aktivt och med ett socialt engagemang ska uppmuntras högt upp i åldrarna.

Nyckelord: Dödlighet, åldersrelaterade sjukdomar, livsstilsfaktorer, genetiska faktorer.

RIASSUNTO ITALIANO

L’aumento dell’aspettativa di vita riveste un notevole interesse nell’ambito scientifico. L'obiettivo principale di questa tesi viene perseguito attraverso l’esame epidemiologico dell’associazione tra stato di salute, lo stile di vita e i fattori genetici da un lato, e probabilità di vivere oltre i 75 anni dall’altro. I dati sono tratti dal *Kungsholmen Project*, uno studio longitudinale su anziani di 75+ anni residenti a Stoccolma, Svezia.

Studio I. La diagnosi di demenza, di malattie cardiovascolari (CVD), o di cancro sono associate a una probabilità 2–3 volte maggiore di morire. Gli anni potenziali di vita persi a causa della demeza sono 3,4, 3,6 per le CVD e 4,4 il cancro. Gli anni medi di sopravvivenza dopo la diagnosi delle malattie sono 4,1 per la demenza, 4,2 per le CVD, e 2,2 per il cancro. Dopo la diagnosi di demenza le donne di 75–84 anni vivono più a lungo rispetto agli uomini coetanei. Infatti, sopravvivono 1,6 anni in più nella fase più grave della malattia.

Studio II. I risultati suggeriscono che le variazioni genetiche dell’*APOE* hanno un effetto opposto sulla sopravvivenza degli uomini e delle donne. Confrontato con il genotipo ε3ε3, l'allele ε2 è un fattore protettivo della mortalità (40% più basso) per le donne, ma non per gli uomini. L'allele ε4, invece, è un fattore di rischio di morte (50% più elevato) solo tra gli uomini. L’aumento del rischio di morte per le persone portatrici dell’allele ε4 è dovuto maggiormente allo sviluppo della demenza, ma non delle malattie cardiovascolari.

Studio III. Essere attivi e avere uno stile di vita sano anche a 75 anni può fare la differenza. Le persone che fanno attività fisica almeno una volta al mese hanno una aspettativa mediana di vita più lunga di circa 2 anni, rispetto alle persone sedentarie. L’aspettativa mediana di vita per i non fumatori che hanno una vita attiva è di 5 anni più lunga rispetto alle persone con una vita sedentaria e uno stile di vita non sano. Vita attiva e buone abitudini producono l’effetto di allungamento della vita anche su persone di 85 anni e in generale sui malati.

Studio IV. I fattori genetici hanno un ruolo rilevante nella sopravvivenza dopo i 75 anni. Variazioni genetiche in 4 diversi geni (*APOC1, APOE, IDE* e *PI3K*) sono associate a una mortalità maggiore del 12–20%. Tuttavia i partecipanti con un profilo genetico sfavorevole (portatori di almeno un allele associato con la mortalità) ma con uno stile di vita sano hanno un rischio di morte circa 70% più basso. Inoltre, la combinazione di un profilo genetico favorevole (assenza degli alleli associate con la mortalità) con uno stile di vita sano diminuisce dell’80% il rischio di mortalità e allunga di 6 anni la durata mediana della vita rispetto a quella delle persone con un profilo genetico sfavorevole e uno stile di vita non sano.

In **conclusione**, la probabilità di sopravvivere oltre i 75 anni risulta fortemente associata con lo stato di salute, lo stile di vita e i fattori genetici. Questi risultati potrebbero favorire la valutazione prognostica della durata generale delle tre malattie sopra nominate. Essi enfatizzano che lo stadio più grave della demenza e quello in cui le persone, in particolare le donne, vivono a lungo. Inoltre, le variazioni alleliche nei geni sono associate ad un rischio di mortalità più elevato. Tuttavia l'effetto della combinazione della predisposizione genetica sfavorevole con fattori ambientali può comportare l'attenuazione del rischio di mortalità, in quanto le persone con una predisposizione sfavorevole possono ridurre il rischio di mortalità modificando il loro stile di vita. Pertanto, gli sforzi per promuovere la cessazione del fumo, l’attività fisica e l'impegno sociale devono essere continuati anche in età avanzata.

Parole chiave: Mortalità, malattie croniche, stile di vita, fattori genetici.

LIST OF ABBREVIATIONS

ACE	Angiotensin I-converting enzyme
AD	Alzheimer's disease
APOA	Apolipoprotein A
APOB	Apolipoprotein B
APOC	Apolipoprotein C
APOE	Apolipoprotein E
ATC	Anatomical Therapeutic Chemical
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CAIDE	Cardiovascular Risk Factors, Aging, and Dementia
CI	Confident interval
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition Criteria
FINE	Finnish, Italian, Netherlands, Elderly study
FTO	The fat mass and obesity associated gene
GWAS	Genome-wide association study
HDL	High-density lipoprotein
HMG-CoARI	Hydroxy-methyl-glutaryl-CoA reductase
HR	Hazard ratio
HPA	Hypothalamic-pituitary-adrenal
ICD	International Classification of Disease
IDE	Insulin-degrading enzyme
IHCD	Ischemic heart and cerebrovascular disease
IGF-1	Insulin-like growth factor 1
IL-6	Interleukin 6
iPLEX	Increased plexing efficiency and flexibility
LDL	Low-density lipoprotein
LE	Life expectancy
LIPC	Lipase
LPL	Lipoprotein lipase
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MAR	Missing at random
MICE	Multivariate Imputation by Chained Equations
MMSE	Mini-Mental State Examination
MTHFR	Methylenetetrahydrofolate reductase
OD	Other types of dementia
PA	Physical activity
PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol 3-kinases
PON1	Paraoxonase 1
RR	Relative risk
SENECA	Survey in Europe on Nutrition and the Elderly; a Concerted Action
SES	Socioeconomic status
SEI	Swedish Socioeconomic Classification System
SNACK	Swedish National Study of Aging and Care in Kungsholmen
SNP	Single nucleotide polymorphism
VaD	Vascular dementia
WHO	World Health Organization

LIST OF PUBLICATIONS

This doctoral thesis is based on the following original papers, which are referred to in the text by their Roman numerals.

I. **Rizzuto D**, Bellocco R, Kivipelto M, Clerici F, Wimo A, Fratiglioni L. Dementia after Age 75: survival in different severity stages and years of life lost. *Current Alzheimer Research* 2012, 9, 795-800.

II. Rosvall L[*], **Rizzuto D**[*], Wang H-X, Winblad B, Graff C, Fratiglioni L. *APOE*-related mortality: Effect of dementia, cardiovascular disease and gender. *Neurobiology of Aging* 2009; 30(10).

III. **Rizzuto D**, Orsini N, Qiu C, Wang H-X, Fratiglioni L. Lifestyle, social factors, and survival after age 75: population based study. *BMJ* 2012, 345: e5568.

IV. **Rizzuto D**, Keller L, Orsini N, Graff C, Bäckman L, Bellocco R, Wang H-X, Fratiglioni L. Hunting for human longevity genes: Interaction with lifestyle factors. *Manuscript*.

[*]These authors contributed equally to the work

CONTENTS

... fructum ferent etiam in senectute, sucosi et vegeti erunt

(Psalmus, 92:15)

1 INTRODUCTION

The oldest person ever, according to verifiable records, was a French woman called Jeanne Calment, who died in 1997 at the age of 122 years and 164 days.[1] Such exceptionally long lives always make us wonder: What is the secret? Does it lie in the genes? What makes the difference? Is it the place where people live or the way they live? Is it something they do or something they do not do? Scientists who study aging say that the secret probably lies in several factors related to both heredity and environment. This introduction provides an overview of the research on longevity, showing the major pieces already in place and those that are still missing in the challenging puzzle of aging and longevity.

1.1 AGING

The fact is that we all age. Independently of age, whether young or old, we are aging. That is the simplest reality that each human being has to deal with and it is part of our life. Distinctions may be made between *chronological aging* and *biological aging or senescence*. Annual birthday celebrations mark the passage of chronological age, whereas senescence is the progressive, cumulative deterioration in function or loss of physiological capacity associated with greater chronological age.[2] However, chronological age does not correlate perfectly with functional age; i.e., two people may be the same age but differ in their mental and physical capacities.

When does old age begin? There is no single age at which we can say that people cross the threshold into *old age*. The process of aging is highly complex, begins at the time of birth, and continues until death. One commonly accepted definition is that people aged 65 and older are *elderly*. Divisions are sometimes made between the young old (65–74 years of age), the middle old (75–84 years of age), and the oldest old (85 years of age and older). Recently, a new phase has been introduced: *the fourth age*, which refers to that period at the end of life when people have completely lost their independence. The fourth age is a direct continuation of the third age, during which chronologically old people still live an independent life.[3]

1.1.1 Magnitude and speed of population aging

The older population (people 60 years and older) is growing at a considerably faster rate than the world's total population (**Figure 1**).[4] Until 1975 the average annual growth rate of the number of persons aged 60 and older was similar to the rate of growth for the total population (about 1.8%). Since then, the annual growth rate of the older population has increased faster than that of the total population and is now around 2.6%–more than twice that of the total population (1.2%). By 2025–2030, projections indicate that the population of older people might be growing about 4 times as rapidly as the total population.[4] Because of slowing birth rate, since 1975 the annual growth rate of the total population has been declining. This decline is projected to continue in the coming years, but the pace of the future change is uncertain. Currently, it is estimated that the annual growth rate of the total population will be less than 1% by 2020. This means that world population will continue to grow in the 21st century, but at a slower rate than in the recent past.

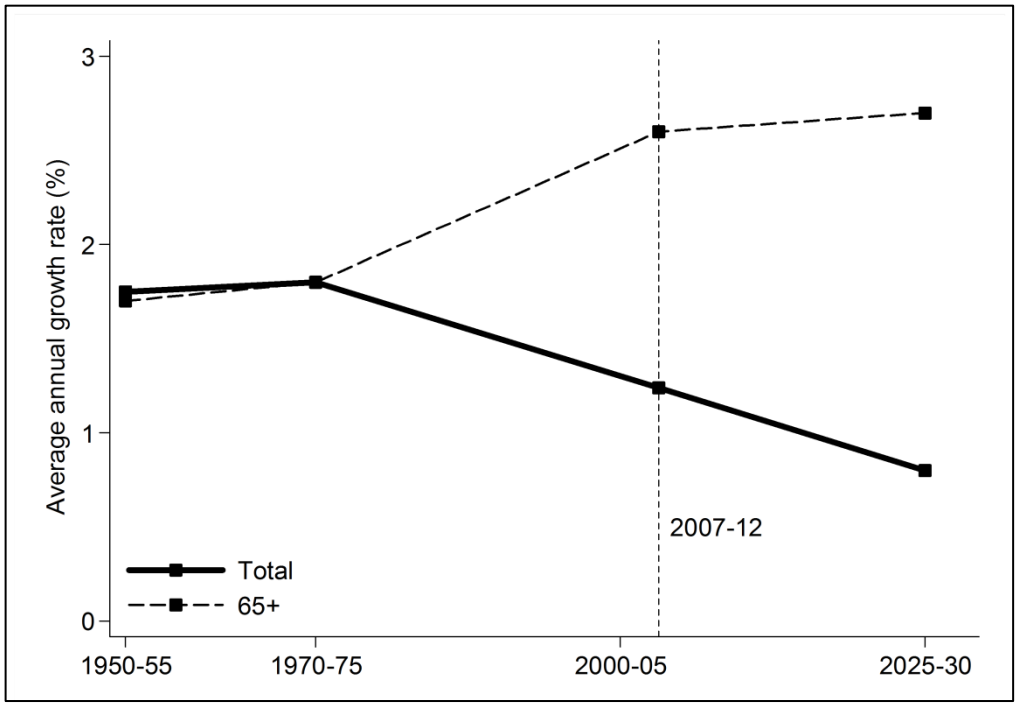


Figure 1 Average annual growth rate of the world's total population and the population aged 60 and older, 1950–2050. Source of data: United Nations Department of Economic and Social Affairs, Population Division

In absolute terms, there were more than 860 million older people in 2010, over 3 times more than in 1950. Over the first half of the current century, the global population aged

60 and older is projected to increase 3-fold, reaching 2 billion in 2050.[4] **Figure 2** shows the steady rise in the proportion of older persons, from 8% in 1950 to 10% in 1990 and 12% in 2010. This proportion is expected to reach 22% in 2050 (Source of data: United Nations Department of Economic and Social Affairs, Population Division).

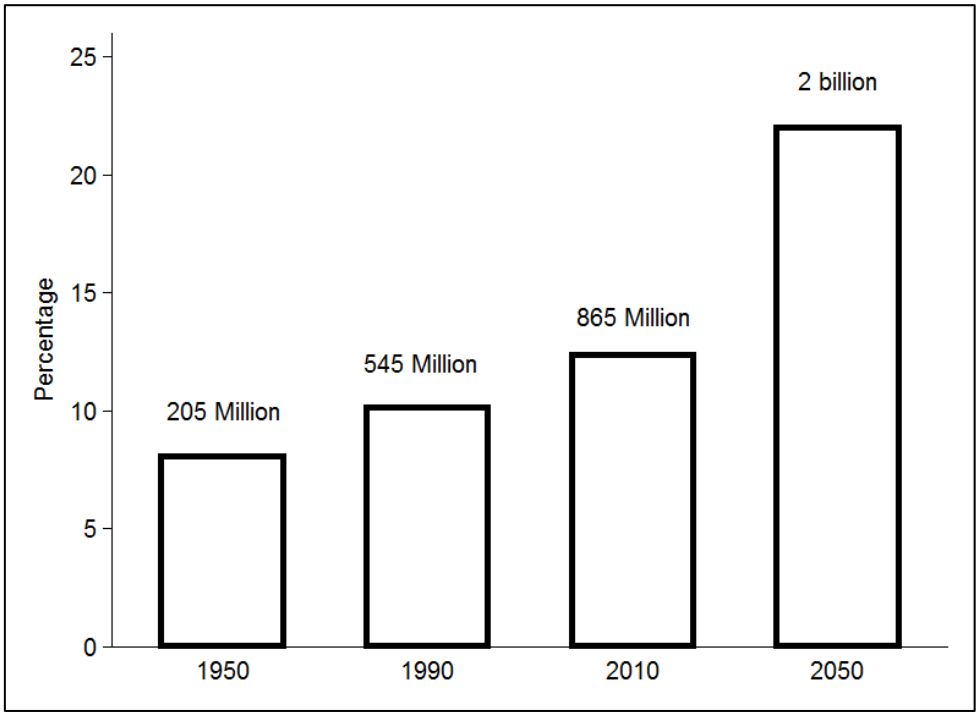


Figure 2 Percentage of the population 60 years and older worldwide, 1950-2050. Source of data: United Nations Department of Economic and Social Affairs, Population Division

Currently, Europe has the highest proportion of older persons of all the continents.[4] Although the highest proportion of older people is found in the more developed countries, the annual growth rate of this age group is higher in the less developed countries. Indeed, by 2050, nearly 80% of the world's older population is expected to live in developing countries.[5]

1.1.2 Demographic transition

Population age structures change when mortality and fertility rates change. Such changes in mortality and fertility rates are known as the demographic transition.[6] The demographic transition that we are experiencing is characterized by the change from high to low levels of both fertility and mortality rates. The transition involves four stages.

The graph in **Figure 3** shows an example of population changes in the Swedish population. In 1750, Sweden was a typical European agrarian country in stage 1 of the demographic transition. It had high annual crude birth (36 per 1000 people) and death rates (27 per 1000 people) and high rates of infectious diseases. The population was subject to periodic famine and infant mortality was high. Sweden transitioned to stage 2 between 1750 and 1850. This period was characterized by a falling death rate due to a dramatic reduction in infant and childhood mortality. The birth rate remained high and may even have increased because women were healthier. After 1850 the birth rate moved downward, catching up with the death rate, and Sweden entered stage 3. This reduction in the death rate resulted from Pasteur's discoveries at the end of the nineteenth century.[7] Moreover, Sweden introduced government-run social security programs between 1930 and 1940, including national health insurance, employee medial insurance, maternity welfare, housing allowances, nursery schools, a children's health system, and free school meals. The government also invested in informational programs on nutrition and health.[8] In stage 4 of the demographic transition, birth and the death rates are again similar but stabilize at relatively low levels (Source of data: Statistics Sweden).

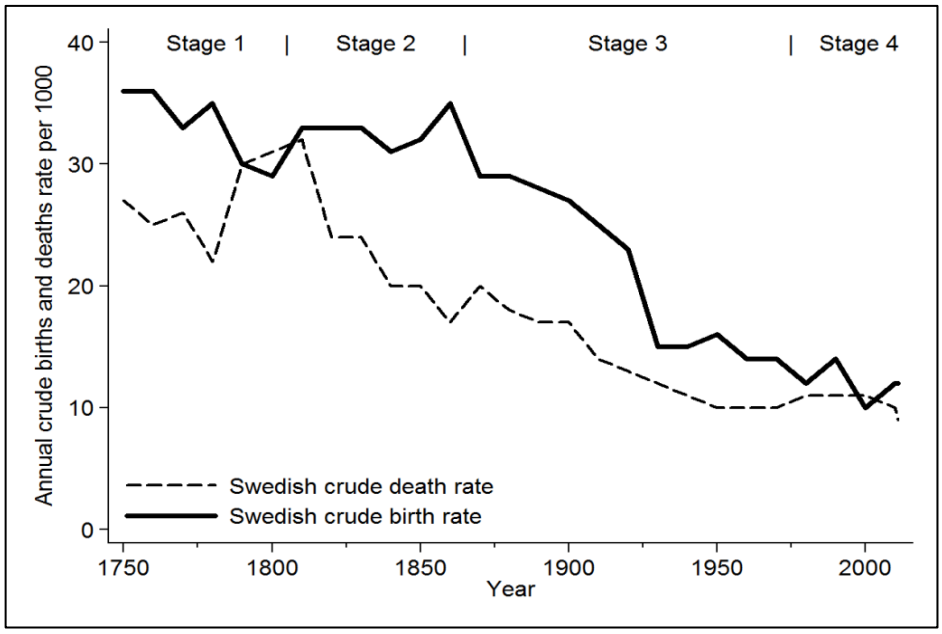


Figure 3 The demographic transition in Sweden: annual crude birth and death rates (per 1000) from 1750 to 2011. Source of data: Statistics Sweden

Population pyramids for Sweden in 2011 and 2050 are shown in **Figure 4**. The pyramids depict the number of men (left) and women (right) in thousands in each age group. The shift in distribution towards older ages by 2050 is readily apparent. The shape of

Sweden's age-sex pyramids is typical of countries that have already undergone the demographic transition from high to low mortality and fertility rates. Both pyramids also show the strong preponderance of women over men in later life (Source of data: Statistics Sweden).

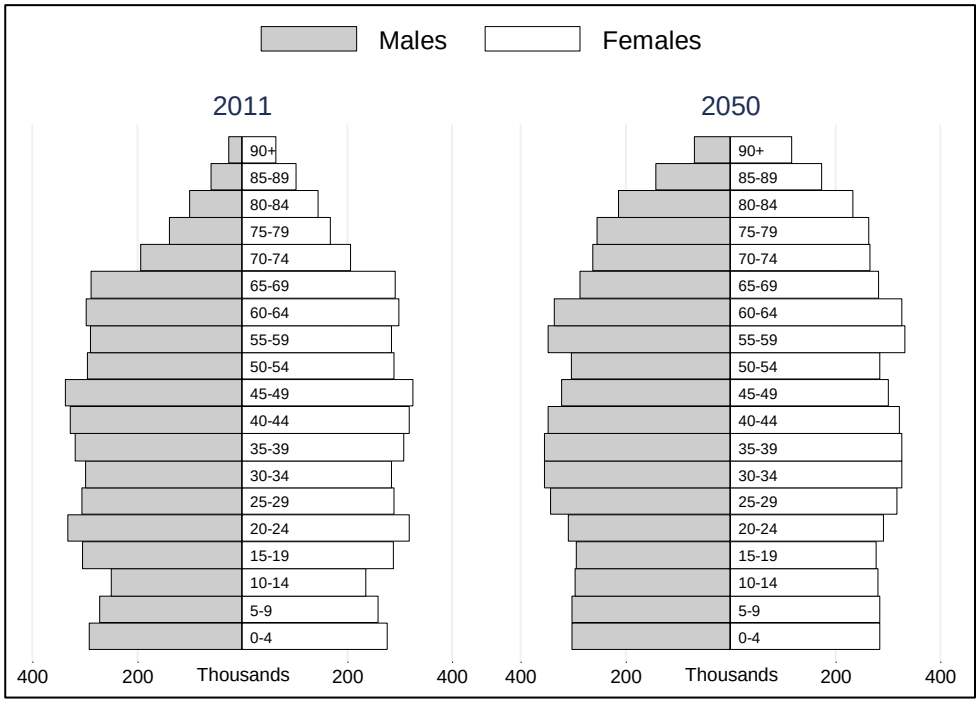


Figure 4 Swedish age-sex pyramid: absolute numbers of inhabitants in Sweden in 2011 and 2050. Source of data: Statistics Sweden

1.1.3 Life expectancy

Life expectancy (LE) is the average number of years that a person at a specific age can expect to live, assuming that age-specific mortality levels remain constant.

Over the last 50 years, improvements in standards of living (income, nutrition, education) and health care (curative and preventive medicine) have resulted in a significant increase in life expectancy for the adult and elderly population. LE continues to rise even in countries like Sweden where the overall average life expectancy in 2012 is 81.9 years of age. According to Statistics Sweden, a Swedish boy born in 2012 can expect to live 80.0 years, and a girl, 83.8 years.

The increase in life expectancy is a relatively new phenomenon in the history of the humanity. Although there have been always single individuals who reached old ages, it is

only during the last 3 to 4 decades that many people have the chance to live to a very advanced age.

This phenomenon in Sweden is illustrated in **Figure 5**. The number of centenarians was extremely low in the middle of the twentieth century. By 1970 there were 127 centenarians, a number that increased to 1700 in 2011. Most centenarians are women.

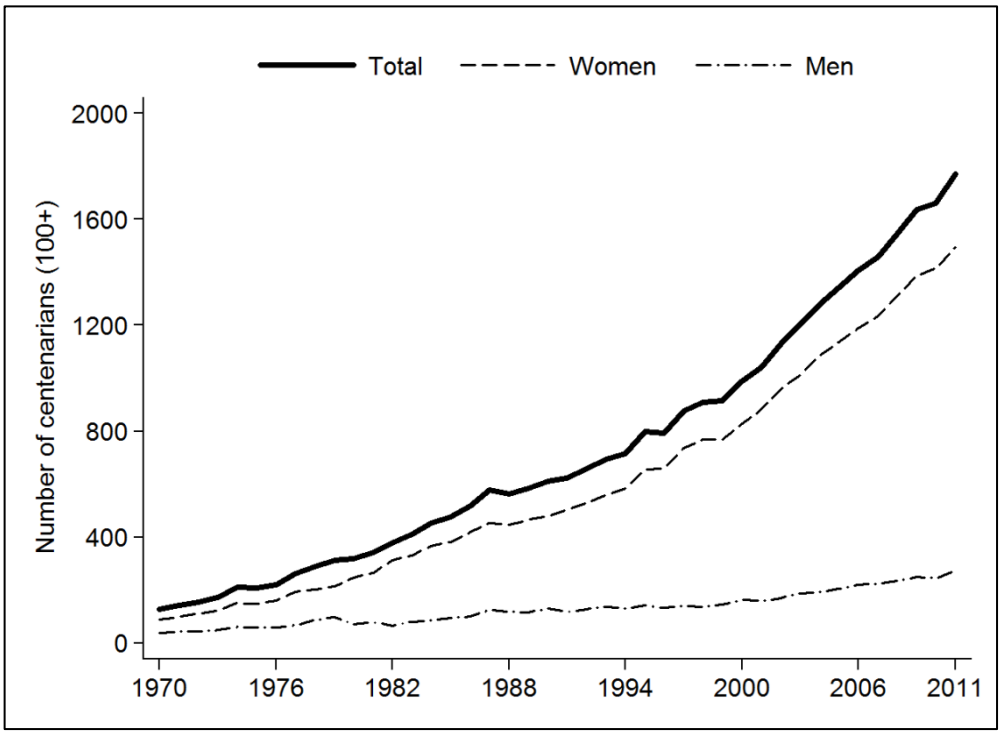


Figure 5 Increased in the number of the centenarians (100+) in Sweden. Source of data: Statistics Sweden

Has the average life expectancy of humans approached its limit? Since the increase in life expectancy has been linear for the past centuries, life expectancy trajectories do not appear to be approaching a maximum.[9] Indeed, a study by Christensen et al., 2009,[10] forecasts that more than 50% of babies born since 2000 in most western countries and Japan will become centenarians if the life expectancy increases over the past 2 centuries continue through the 21st century.

1.1.4 Health of the aging population

Despite the positive nature of the trend in life expectancy, we must still face the major question of how to live, not only longer but also healthier.

In Europe, as in many other places, future health status will be strongly influenced by population aging. Thus, there is an urgent need to better understand the aging process with the ultimate goal of improving the health and quality of life of elderly people. For these reasons, several years ago researchers in various countries started population-based studies that follow middle-aged and elderly people to trace changes in their health status over time and identify factors relevant to better health and quality of life in old age.

The 20th century saw an epidemiological transition in which a reduction in many epidemic infectious diseases was counterbalanced by a steady rise in chronic conditions. Many of these conditions are associated with important changes in lifestyle[11] and are the consequence of accumulated exposure to risk factors over a person's lifetime.[12,13] Thus is not unusual for a person who has reached retirement age to have at least 2 chronic conditions,[14] and such diseases— i.e. cardiovascular disease, dementia, and cancer—have replaced infectious diseases as the main causes of death today. Moreover, these public health problems remain largely unresolved. Given the current situation and trends, it seems likely the future of health care will be dominated by the challenge of complex chronic disorders, especially among elderly people.

Currently, there are several theories about what is happening and will happen to the health of elderly people as the population ages. The *compression of morbidity* theory was put forth by James Fries in 1980. Fries' hypothesis is that the burden of lifetime illness may be compressed into a shorter period before the time of death if the age of onset of the first chronic infirmity can be postponed.[15] This hypothesis contrasts with the view that if medical progress extends life for persons with chronic and disabling conditions but does not reduce the incidence of these conditions, the health of the population will deteriorate (the theory of *expansion of morbidity*).[16] In 1982, Manton proposed a position between the 2 outlined above.[17] His hypothesis, known as *dynamic equilibrium*, is that alongside the reduction in mortality there will be a reduction in the rate of deterioration of the body's vital organ systems. This could result in more diseases in the population, but the diseases will be less severe.

1.1.5 Models of aging and longevity

Several models with different outcomes have been formulated to attempt to explain aging process. These models have different outcomes and categorize possible determinants differently. In 2004 Crimmins and Seeman proposed a heuristic model of several

potentially interacting pathways of interest in understanding health-related outcomes linked to aging.[18] Health outcomes included mortality, physical and cognitive functioning, and cardiovascular disease. Kirkwood, in 2005, proposed a model in which a lifelong accumulation of molecular damage underlies the aging process.[19] As cell defects accumulate, the body will express such damage with symptoms, disease, and functional impairment. The degree of accumulation is accelerated by stress, environmental risk factors, and poor nutrition, and modulated by healthy lifestyle and healthy nutrition. According to Ryff and Singer, 2009, health in aging is the result of numerous influences that can be divided into 3 broad categories: socio-structural factors, individual-level factors, and biological factors.[20]

In spite of some differences, all models agree that the individual is the central figure in the model, together with his/her biological background (including genetics) and his/her life-long exposure to different risk and protective factors. This life-long process brings an accumulation of different degrees of damage to 1 or more organs, which accelerates the aging process and promotes functional impairment and morbidity.

1.2 DETERMINANTS OF LONGEVITY

The word longevity (long life), comes from the Latin word *longaevitās*. Longevity is the capability to survive beyond the species-specific average age at death.[21] This definition involves not only the individual ability to achieve old age but also population-level mortality, measured in this case by the mean age at death of the population (or life expectancy). Alternative definitions of longevity refer to the period between birth and death of an individual; that is, the maximum lifespan actually achieved.

Current evidence from several studies indicates that longevity reflects a variety of underlying factors, including genetics, environmental, and medical factors.[22] It also contains a stochastic component that results from the interaction between individual chances of surviving and unpredictable events that occur throughout the life course.[23] It is commonly accepted that genetic background explains around 20–30%[24] of the variability in longevity, the stochastic component around 20%,[23] and the environmental factors the remaining 50–60% .

It is possible to hypothesize that there are multiple ways to achieve exceptional longevity by combining these 3 components.[21] However, probably none of these factors is necessary or sufficient to determine the aging phenotype at the individual level.

The identification of the possible determinants of human longevity is still an open debate. Most previous studies that searched for the determinants of longevity were carried out within specific research fields such as genetics, medicine, or demography. The major consequence of this discipline-specific approach is that often only a selected number of variables are considered which renders the search of determinants of longevity limited. Nevertheless, a critical review of the existing literature provides useful indications of the possible candidate determinants of longevity.

1.2.1 Gender

Gender is clearly the main factor to take into account when investigating differential longevity. It is widely recognized that women generally live longer than men. In 2011, LE was 3.9 years higher in Swedish women than in Swedish men. With the exception of few countries, LE at birth is higher for females than males worldwide, as shown in **Figure 6**. In the figure, the difference in LE between genders (gender gap) is also reported in number of years.

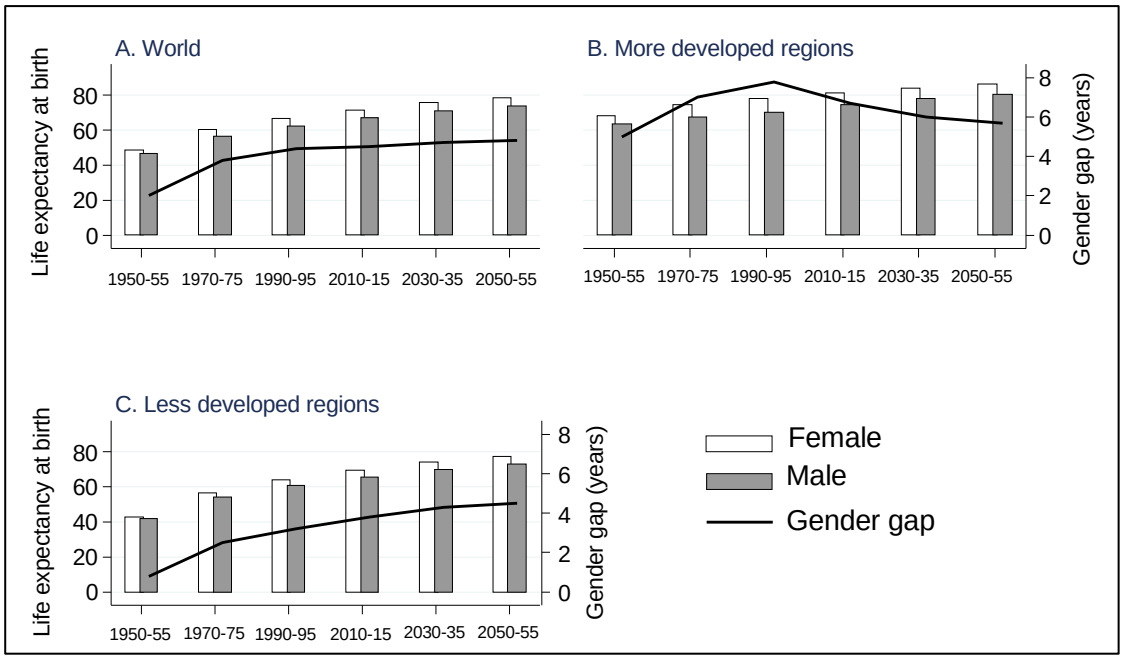


Figure 6 Male and female life expectancy at birth and gender gap in life expectancy, 1950–2055. Source of data: United Nations Department of Economic and Social Affairs, Population Division

The reasons for this gender gap are multifactorial and not completely understood.[25-29] Several hypotheses have been proposed to explain sex difference in longevity. Major putative factors fall into 2 general categories: external (social, cultural, environmental, and behavioral), and constitutional (biological and genetic) factors. One hypothesis attributes the gender gap to a larger participation of men in labor force.[30] If this hypothesis is valid, nowadays we might expect a decrease in differential mortality rate because of improved gender equality. However, women still outlive men today. A second hypothesis is that women live longer because they are exposed to a smaller number of risk behaviors. Although a gender gap in risk behaviors may have existed in the past, it seems to have closed today at least for major risk factors such as smoking. Indeed, according to the Statistics Sweden, in 2008 the proportion of smokers was higher among women than men, at all ages (**Figure 7**). Therefore, it is unlikely that lifestyle may fully explain the gap.

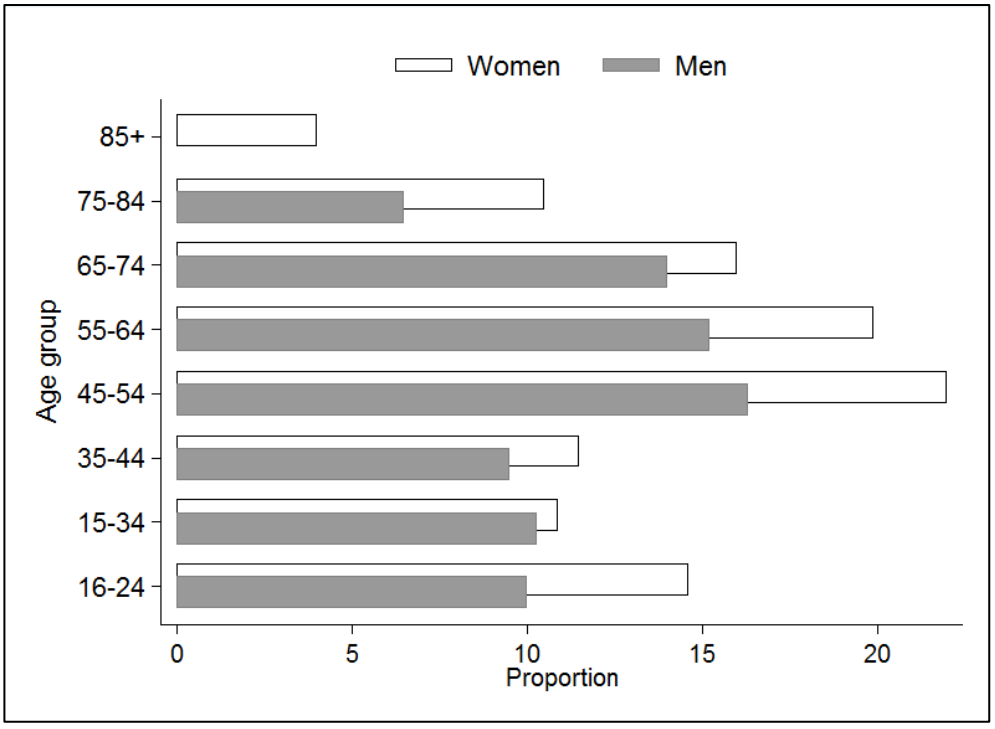


Figure 7 Male and female proportion of daily smokers in 2008. Source of data: Statistics Sweden, Living condition survey

As several studies have shown that female animals of most species live longer than male animals, an explanation of the gender difference in human LE might lie deep in our biology.[29] In 2006, Austad wrote that "Females do not live longer than males because they age slowly, females live longer because they are more robust at every age."[26] He

drew this conclusion by dividing the U.S. population into groups by age and comparing the rates of death in men and women for all the most frequent causes of mortality.

It has been hypothesized that chromosomal differences between men and women may affect their longevity. After conception in females 1 X chromosome gene is randomly inactivated in each cell. X chromosome inactivation typically protects against a double dose of X expression in females; however, it also protects females against disadvantageous genes on 1 X chromosome. Because women have 2 X chromosomes, a female with an abnormal gene on 1 of her X chromosomes can use the normal gene on the other and thereby avoid the expression of disease (although she is still a carrier of the defect). Men, in contrast, have 1 X chromosome and 1 Y chromosome, and so they cannot rely on an alternative chromosome if a gene on 1 of the sex chromosomes is defective.[26,31,32]

Moreover, genetic sex differences are not limited to the nuclear genome. Mitochondria are inherited from human mothers only, and, consequently, it has been proposed that the mitochondrial genome is optimized for function with the female genome through natural selection that predominantly acts on mitochondrial-nuclear genome interactions in females.[32] This optimization of mitochondrial function in cells in females could confer a life-span advantage given that mitochondrial dysfunction has been implicated in aging[33] and disease.[34,35]

Experts suspect that gender differences in mortality patterns may be influenced at least in part by sex hormones. After conception, different circulating concentrations of sex hormones (such as estrogen, progesterone, and testosterone) underlie many of the physiological differences between the sexes. Hormonal influences on female biology at the organizational level in early fetal and childhood development, as well as after puberty, lead to favorable differences in immune function, oxidative stress and antioxidant status, lipoprotein metabolism, lipid storage and metabolism, the hypothalamic-pituitary-adrenal (HPA) axis stress response, and the ability of cells in females to maintain integrity in the face of several stresses. A combination of these factors may play a role in female life expectancy advantage.[32]

In conclusion, in spite of increasing number of studies on aging and longevity, at present we still lack strong evidence to support any of the proposed hypotheses. We should also consider that all these hypotheses may be not mutually exclusive. Instead, various gender-related mechanisms may contribute to the gender gap in longevity.

1.2.2 Socioeconomic factors

Throughout the twentieth century, adult mortality rates have exhibited impressive declines in all high-income countries. The latter half of the twentieth century was, however, characterized by well-documented differences in adult mortality rates across categories of socioeconomic status (SES).[36-41] Many studies show that mortality inequalities related to SES tend to be narrower among older age groups,[42-45] although they persist to 80 years of age and older.[42,46-51] Among the 4 major components of SES (education, occupation, income, and wealth), education, often expressed in terms of years of schooling, is 1 of the most frequently used when studying socioeconomic differences in adult mortality.[52] There are several reasons for this choice. First, educational attainment is often completed relatively early in adult life and usually remains constant throughout adulthood. In contrast, occupational status, income level, and the accumulation of wealth may vary in considerable ways throughout the life course. For that reason, education may represent a proxy for socioeconomic or environmental influences in childhood.[53] Indeed, education is a good indicator of the social backgrounds of children as well as their level of knowledge and skills.[54] Second, because of international similarities in educational systems, use of educational attainment as a proxy for SES makes the results of studies from different countries easier to compare.[48] Finally, education typically precedes occupational status, income, and the accumulation of the wealth; therefore it may represent a proxy for all these factors.

In all countries studied, death rates are higher in those with less education than those with more education. This pattern has been established in comparative works examining Scandinavian nations (Denmark, Finland, Norway, and Sweden); western and southern countries (Austria, Belgium, Bulgaria, England, France, Italy, Hungary, the Netherlands, Spain, and Switzerland); countries in the former Soviet Union (Estonia, Lithuania, and Russia); and countries in North America (Canada and the United States).[38,48,50,55-63]

Socioeconomic inequalities in mortality in old age are likely to be a product of lifetime exposures to adverse social and occupational exposures.[64] Education helps individuals acquire resources and attitude that influence health over the life course and, ultimately, how and when individuals die.

Several frameworks have been proposed as possible explanations of socioeconomic inequalities in mortality: socioeconomic attainment, health behavior, and psychological resources.

The association between mortality and educational level might be explained by material factors (socioeconomic attainment). Because people with more education tend to have higher incomes, they can use the economic resources available to them to pay for better life conditions such as nutrition and housing and to live in safer neighborhoods.[52] A longitudinal Dutch study[65] showed that people with financial problems, adverse income situations, or unemployment due to disability have higher mortality after 5 years of follow-up than those without such disadvantages. In turn, higher family income is associated with lower mortality risk at all ages.[52] In addition to income, occupational status could explain the association between education and mortality. Educated individuals are more likely to have regular employment, a high status occupation, and more creatively oriented jobs. Studies show that all these factors are associated with better health and lower mortality risks.[66-68]

Other studies show that behavioral factors (health behaviors) partially explain socioeconomic inequalities in mortality. There is a well-established increased risk of adopting unhealthy lifestyles (smoking, obesity, physical inactivity, and high alcohol intake) among people with lower SES.[69,70] However, Latz et al., 1998,[71] found that lifestyle factors explain no more than 12–13% of the predictive effect of income on mortality.

Finally, researchers suggest that another possible mechanism through which high education level improves health and reduces mortality are social psychological resources. Highly educated people may have access to other highly educated individuals, who can provide help and advice in times of need. For example, Montez et al., 2009,[72] showed that educational differences in mortality are more marked among unmarried than married men. It has also been found that lower parental education is significantly associated with multiple metabolic risks (i.e., higher insulin, glucose, and cholesterol level) and cumulative risk of cardiovascular health problems in adolescence.[73]

1.2.3 Lifestyle factors

People who live in Sardinia (Italy), Okinawa (Japan), and Loma Linda (California) have a significantly longer LE than people in other parts of the developed world. A study of people in these areas found that these populations share common lifestyle characteristics that may explain their longevity: no smoking; constant moderate physical activity; social

engagement; and a diet rich in vegetables, fruits, and whole grains are some of the common characteristics.[74]

Indeed, according to the World Health Organization (WHO), the major causes of death among the high-income countries in 2008 were primarily related to lifestyle (**Table 1**). Moreover, several previous studies have reported that longevity and other health-related outcomes are associated with healthy lifestyle factors.[75-95]

Table 1 The 10 leading causes of death in high-income countries (2008). Source: WHO

Causes of death	Deaths in millions	% of deaths
Ischemic heart disease	1.42	15.6%
Stroke and other cerebrovascular disease	0.79	8.7%
Tracheal, bronchial, and lung cancers	0.54	5.9%
Alzheimer and other dementias	0.37	4.1%
Lower respiratory infections	0.35	3.8%
Chronic obstructive pulmonary disease	0.32	3.5%
Colon and rectal cancers	0.30	3.3%
Diabetes mellitus	0.24	2.6%
Hypertensive heart disease	0.21	2.3%
Breast cancer	0.17	1.9%

1.2.3.1 Tobacco use

Tobacco use is a global public-health concern. The use of tobacco is a risk factor for at least 6 of the 10 leading cause of death worldwide: ischemic heart disease, cerebrovascular disease; tracheal, bronchial, and lung cancers; lower respiratory infections; and chronic obstructive pulmonary disease. Worldwide, tobacco use causes more than 5 million deaths per year, and current trends show that tobacco use will cause more than 8 million deaths annually by 2030.[96] For every person who dies because of a smoking-related disease, 20 more will experience at least 1 serious illness from smoking.[97] The deleterious effects remain even after quitting, although the relative risks decline over time. Doll et al., 2004,[98] compared mortality risk by smoking habits in male British doctors in a 50-year follow-up study. They found that smokers died on average 10 years earlier than non-smokers. Smoking cessation at age 60, 50, 40, and 30 years increased life expectancy of approximately 3, 6, 9, and 10 years.

Studies on the association between smoking habits and survival in elderly populations have yielded inconsistent results. Whereas studies that examined this topic in 60 years and older individuals consistently found that smoking predicts higher mortality,[81,82,84,88,89,91,94,99,100] studies on 80 year and older individuals are not consistent. In a 2008 study of 6084 city dwellers aged 80 years and older, Dupre et al.,[92] found that current or past smokers were significantly less likely to be among the oldest adults in the population. Other studies among nonagenarians[86,101] and centenarians[87] have not found a significant association between current and/or past smoking habits and longevity. One possible explanation is that nonagenarians and centenarians may have genetic or environmental characteristics that protect them against the well-known harmful effects of tobacco smoking.

1.2.3.2 Body mass index

The impact of body mass index (BMI) on mortality in the growing elderly population is still controversial.[102]

Obesity is another one leading preventable cause of death worldwide.[103] In Europe, a million deaths (7.7%) each year are attributed to excess weight.[104,105] On average, obesity reduces life expectancy by 6 to 7 years.[106-108] However, the harmful effects of overweight/obesity on survival are weaker in 65 and older than younger adults.[109-111] A review of 13 prospective studies of people aged 65 years and older concluded that evidence did not support mild-to-moderate overweight (BMI range from 20 to 27) as a risk factor for all-cause mortality.[112] In addition, only few studies found a significant association between BMI over 27 and mortality, this association seems to disappear for individuals aged 75 years and older.[112] The weak association between BMI and mortality has been found also in a study on Swedish centenarians.[87] Furthermore, an inverse association between increased BMI and 5-year mortality has been reported among 65 to 84 year-old male participants in the Finland, Italy, Netherlands Elderly Study (FINE).[85] Similar results have been found in Danish nonagenarians, among whom those with a BMI greater than 28 had the lowest mortality.[86] In a more recent review and meta-analysis, which included 32 studies, authors concluded that BMI in overweight range is not associated with an increased mortality, and that a BMI in the obese range is only associated with a modest increase (about 10%) in mortality risk in the elderly.[102]

It is possible that no association between high BMI and mortality has been found among individuals aged 75 years and older because those who were obese (BMI 30 or higher) may already have died. It has been suggested that the positive association between increased BMI in elderly people and survival is caused by fat stores that are different to and metabolically better than fat stores in young adults.[113] Indeed, there is evidence that the type and distribution of fat stores in older people differs from those in young adults.[114,115]

A number of studies have reported increased mortality among individuals in the lowest BMI categories.[102,108,110,112,116,117] The mortality rate tends to increase when BMI falls below the range of 19–23. Being underweight was found to be associated whit loss of peripheral and respiratory muscles[118] and it might increase vulnerability to acute disease,[119] this might explain the association between underweight and increased mortality. Moreover the reverse causation is a concern in studies of lower BMI and mortality, the possibility that pre-existing illnesses or condition associated with increased mortality lead to loss of body weight.[102]

1.2.3.3 Alcohol consumption

Together with tobacco, smoking, and obesity, over-consumption of alcohol is listed among the leading preventable causes of death worldwide.[103] Several studies have found a U- or J-shape association between alcohol intake and mortality. Heavy drinkers but also abstainers have higher mortality than light and moderate drinkers.[120-125] In a recent report, researchers found that light wine consumption was associated with a 5-year increase in life expectancy.[126]

In older people, however, the relationship between alcohol consumption and mortality is not so clear.[127] Four studies among elderly people have reported an increased mortality risk associated with increased alcohol intake.[81,125,128,129]An average weekly consumption of 14 or more drinks gave older men—but not older women—a higher risk of mortality than non-drinkers of the same age.[128] Doll et al, 1994,[129] reported that male doctors who consumed 29 or more drinks a week had a higher risk of mortality than male doctors who did not drink. A U-shape relationship has been found between alcohol consumption and mortality risk in elderly Danes. Abstainers and heavy drinkers (28 drinks or more a week for women, 69 drinks or more a week for men) had a higher risk of dying than light drinkers.[125] Finally, mortality risk was found to be 2 times higher among elderly people

who consumed more than 14 drinks per week than among abstainers.[81] In contrast, 4 studies have reported an inverse association between mortality and increased alcohol intake in older people.[82,99,130,131] People aged 60–79 years in the United States who reported a weekly consumption of 28 drinks or more were found to have a lower risk of mortality than nondrinkers.[130] Elderly Australian men, but not women, who had this same level of alcohol consumption, had survived longer than non-drinkers.[99] A study of an elderly Mediterranean population found that those who drank moderately or heavily had a lower risk of mortality than nondrinkers.[82] Finally, McCaul et al., 2010,[131] investigated the effect of alcohol intake on 10-year mortality in men and women over the age of 65. They found that the risk of all-cause mortality was lower in men who consumed up to 4 standard drinks per day and in women who consumed 1 or 2 standard drinks per day than in older adults of the same sex who did not consume alcohol every week. Other studies have not found any association between alcohol intake and mortality.[84,86,94,101,121,122,132-139]

Possible explanations for the inconsistent findings from these studies are the variation in the study-specific cut-points, the choice of the reference group, and likely classification errors. Underreporting of alcohol use could also have prevented the detection of adverse effects. Finally, the survival cohort effect should be also taken into account. People with alcohol-related diseases could die prematurely, leaving a cohort that is less susceptible to the adverse effects of alcohol. Many biological changes should make elderly people more vulnerable to excessive alcohol consumption than adult population. Moreover, elderly people are more likely than younger people to have multiple comorbidities[14] and to use more medication.[127] Alcohol may interact differently with different chronic diseases and with medication to adversely affect health outcomes. Thus, caution is necessary in the interpretation of the inverse association between heavy alcohol intake and mortality.

1.2.3.4 Social network

The influence of social relations on mortality is well documented. A recent meta-analysis of 148 studies confirms that social relationships significantly predict mortality.[140] However, it is unclear if all social relationships are equally beneficial to elderly people or if specific types of relationships are more advantageous. Giles et al., 2005,[141] showed that the beneficial association between social networks and survival may be restricted to relationships with friends and confidents rather than with children and relatives. In a meta-analysis of 53 prospective observational studies of elderly people, marriage or

support from a partner was found be a significant independent predictor of survival; the overall reduction in mortality risk was 9–15%. However, this association was statistically significant in only half the studies.[142]

Social integration may influence survival via several potential mechanisms. For instance, social integration may influence health behaviors.[143-145] It has been demonstrated, for example, that social integration is significantly associated with physical activity.[146] Social relationships may provide resources for adaptive behavioral or neuroendocrine responses to acute or chronic stressors. Although it has been found that physiological activation in response to stressors is beneficial up to a point, excessive activation may have hidden costs.[147] Supportive social relationships can moderate stress responses indirectly; for example through practical assistance in time of need.[148]

1.2.3.5 Physical and leisure-time activity

The majority of the studies that investigate the relationship between leisure activities and health focus mainly on younger or middle-age populations; few reports have examined the association between these factors and survival among elderly people. However, a growing body of empirical evidence suggests that being active and participating in different kinds of leisure activities has a positive effect on survival, even among elderly people.

Physical activity (PA). The connection between PA and mortality has been well studied. Three recent systematic reviews and meta-analyses of observational studies have shown that PA reduces mortality among men and women.[149-151] However, as most of the studies included in these reviews involved people who were 20 and 65 years old, it is unclear whether these results are generalizable to older people. A recent study of 14000 elderly people followed for 28 years showed that any amount of time spent in PA resulted in a lower risk of mortality. This effect was reached even with a level of PA lower than the current guidelines for older people: a minimum of 30 minutes of moderately intensive activity 5 days a week.[152] A population-based cohort study of 2357 men aged 65 and older suggests that regular rigorous exercise decreases mortality risk.[94] Parallel results have been found in the 2600 participants aged 75 years and older enrolled in the Survey in Europe on Nutrition and the Elderly; a Concerted Action (SENECA)[89] and in participants in the Italian Silver Network Home Care Project (2757 people 70 years of age and older

who were followed for at least 1 year).[153] Comparison between these studies is not easy because various definitions of PA and activity levels were used.

Leisure-time activity. Far fewer studies have analyzed the association between activities that have a physical component and mortality in elderly adults. In a study of 2761 American men and women aged 65 and older, researchers found that increased participation in social activities (such as attending church; going to the movies, to the theater, or to sporting events; and playing games) and productive activities (such as gardening, shopping, community work, and cooking) was associated with decreased mortality.[154] In a Swedish cohort of 463 people aged 77 and older, greater participation in solitary activities with a physical component (gardening and other hobbies) was associated with a significant reduction in mortality.[155] In a 6-year follow-up analysis of 2291 people aged 67 to 95 years, a greater overall activity level was associated with reduced mortality. Activities were classified as follows: social activities (visiting family and friends, church-related activities, and sports); solitary activities (hobbies, going to the theater, music, art, reading, and writing); and productive activities (volunteer work, housework, gardening, and yard work).[156] In the Leisure World Cohort Study, 14000 elderly men and women (median age 74 years) were followed for 28 years, and different activities were analyzed in relation to survival. Participation in less physically demanding activities reduced mortality, as did participation in physical activities.[157] These results were confirmed in a Swedish cohort of 1246 people (mean age 75 years).[158] Engagement in hobbies, dancing, organizational activities, and gardening were found to be associated with reduced mortality.

Various pathways between leisure activity and survival have been suggested. Physical activity may effect mortality risk through physiological pathways, such as improving cardiovascular health[159] and functional status.[160] Psychosocial pathway may be involved in the health benefits of activity. Social participation often provides individuals with meaningful roles, greater social networks, and social support, which protect against damaging physiological responses by improving coping abilities and heath behaviors.[161]

1.2.4 Genetic factors

The genetic contribution to longevity and human aging is likely to result from many different genes, each of which makes a modest contribution.[162] Some genes likely affect longevity by increasing predisposition to age-related diseases and early death; other genes are likely to slow the aging process itself, leading to a longer life. We still do not know, however, how genetic factors interact with modifiable behavioral and environmental factors to contribute to longevity.[162]

The genetic contribution to longevity has been estimated using twin studies as well as population based studies (**Table 2**). Most heritability estimates from twin registers range between 20 and 30%,[24,163,164] whereas estimates from population-based studies are slightly lower, ranging from 15 to 25%.[165-167] Evidence shows that genetic influences on longevity may vary by ethnicity.[168] One study shows that African Americans have a lower heritability than European or Caribbean Hispanic populations.[168] In 2006, Hjelmborg et al.,[169] published the results of a study on the genetic influence on human lifespan and how it varies across age, using Nordic twin cohorts (Danish, Finnish, and Swedish). They reported that the genetic effect on lifespan is minimal before age 60 years, but increases thereafter.

Table 2 Estimates of heritability contribution to longevity from different studies

Sample	Heritability
Danish twins[24,163]	20–30%
Swedish twins[164]	33%
Old Order Amish population[165]	25%
Utah Population Database[166]	15%
Framingham Heart Study[167]	16%
Medicare recipients, New York City[168]	
European ancestry	26%
African American	4%
Caribbean Hispanic	29%

To identify genes that influence human longevity, both linkage and association studies have been carried out. Linkage analysis is used to map genetic loci and is based on observations of closely related individuals.[170] These kind of analyses often have the

disadvantage of a lack of availability of multi-generational DNA from long-lived individuals and the complexity of increased lifespan as a trait.[171]

Association studies investigate the relationships of specific variants of candidate genetic with a given phenotype. One type of study design often applied in association studies is the case-control design. These studies are designed to compare frequencies of variation in candidate genes between groups. A proper control group is critical in these studies to avoid the introduction of severe bias. Longitudinal studies, in which a cohort of individuals is followed over time, make it possible to examine incident events in relation to specific genetic variations. So far, in most studies on the effect of genetic variations on longevity, researchers have examined only 1 or at most a few SNPs in the same genes. These variations are often chosen for their possible effect on protein function or level of expression. However, haplotype analyses or "whole gene" analyses, undertaken by tagging SNPs, might increase the possibility of finding an association between variation in genes and longevity. To date, 7 genome-wide association studies (GWAS) on longevity have been performed.[172-178] Genome-wide association studies can be problematic, however, because the massive number of statistical tests performed presents an unprecedented potential for false-positive results.[179]

Using all these approaches, many candidate genes have been investigated in relation to survival or longevity. As yet, however, only a few genetic variants have been found to influence longevity. Apolipoprotein E (*APOE*) is the only gene with common variants that have consistently reported associations with longevity in humans, supposedly via their association with both Alzheimer's and cardiovascular disease.[171] Many initially positive findings between other candidate genes and longevity have not been replicated. Some of the most thoroughly investigated or biologically most plausible candidate genes are listed in **Table 3**.

The discrepancy in the findings of the different studies might be the result of differences in experimental design, sample size, criteria used in selecting subjects, and examination of different alleles in the same gene. In addition, the association between polymorphisms and mortality might have a population-specific component; it may be affected by the population-specific gene pool, gene-environmental interactions, or both. Absence of a statistically significant association does not necessarily mean that there is no association; thus, it is important to continue these studies in larger cohorts to increase our understanding of mechanisms that contribute to increased survival.

In summary the scientific world has shown an increased interest in the aging phenotype, probably as a consequence of the increasing average human lifespan and the growing percentage of elderly people in the population. This thesis attempted to investigate only some of the potential determinants of longevity. In addition to the numerous factors discussed here, others important factors contribute to the human longevity. These include but may not be limited to psychological problems (stress), violent deaths (suicide, homicide, and motor vehicle accidents), and the environment and occupation exposure (air pollution, ultraviolet light, toxic metals/solvents, and food and water supply).

Table 3 Selected human candidate genes involved in survival

Gene names (symbol)	Protein function	Pathway	Study design	Population, reference	
				Positive association	**No association**
Angiotensin I-Converting Enzyme (*ACE*)	Hydrolyses angiotensin I to angiotensin II	Risk factor for cardiovascular diseases	Case-control	Brazilian (European origin),[180] Chinese,[181] Columbian,[182] Croatian and European,[183] English,[184] French,[185,186] German,[187] Italian,[188] Russian[189]	American Caucasians,[190] Chinese,[191] Danish,[192-194] Dutch,[195] European,[196] French,[197] Italian,[198] Korean,[199] Polish,[200] Spanish[201]
			Longitudinal	Chinese,[202] Danish,[203] Dutch[204]	
Apolipoprotein A (*APOA*)	Promotes cholesterol efflux from tissues to the liver for excretion	Risk factor for cardiovascular diseases	Case-control	Italian[205,206]	French[207]
			Longitudinal	Danish[208]	
Apolipoprotein C (*APOC*)	Component of very low density lipoproteins and chylomicrons	Risk factor for cardiovascular diseases	Case-control	English,[184] Finnish,[209] Jerusalem,[210] Russian[211]	
			GWAS study	German[178]	
Apolipoprotein E (*APOE*)	Ligand for the LDL receptor – transportation of cholesterol	Risk factor for cardiovascular and Alzheimer's disease. Stress response	Case-control	Chinese,[212,213] European,[214] Finnish,[209,215,216] French,[185,197] Greek,[217] Italian,[188,218] Jerusalem,[219] Swedish[220]	Brazilian,[221] Columbian,[182] English,[184] Italian,[222] Japanase,[223] Korean[199]
			Longitudinal	American (Utah),[224] Danish,[225,226] Finnish[227] Swedish[228]	Finnish[229,230] Dutch[231]
			GWAS study	Dutch[177]	

Gene names (symbol)	Protein function	Pathway	Study design	Population, reference	
				Positive association	**No association**
Insulin-like growth factor 1 receptor (*IGF1R*)	The receptor of the insulin-like growth factor – stimulator of cell growth	Stress response	Case-control	Italian,[232] Jerusalem[233]	
Interleukin 6 (*IL-6)*	An immune regulatory cytokin–acute phase response; e.g., fever	Inflammation and stress response	Case-control	Danish,[234] Finnish,[235] Italian,[236] Irish[237]	Bulgarian,[238] Finnish,[239] Irish,[240] Italian,[241,242] Japanese[243]
			Longitudinal	Italian[244]	American[245]
Interleukin 10 (*IL-10)*	Anti-inflammatory cytokine	Inflammation, and stress response	Case-control	Finnish,[239] Italian,[246,247] Japanese,[243] Jordanian[248]	Bulgarian,[238] Irish,[240] Italian[241]
Tyrosine hydroxylase (*TH*)	Enzyme responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine	Stress response	Case-control	Italian[249,250]	German[251]
Paraoxonase 1 (*PON1*)	Preserves high-density lipoprotein function and protects low-density lipoprotein from oxidative modification	Stress response and neuro-degenerative diseases	Case-control	French,[252] Italian,[253-255], Various (meta-analysis)[256]	Danish,[257] Irish,[255] Various (meta-analysis)[258]
			GWAS		Various (meta-analysis)[175]

2 AIMS

2.1 GENERAL AIMS

The overall aim of this thesis is to detect the most relevant factors that lead to longer survival after age 75. The thesis will explore the role of health, lifestyle, social environment, and genetic background in the probability of reaching a very advanced age. The major research questions are: What is the impact of the most common chronic diseases in the elderly people on their survival? What effects do lifestyles and social environment have on life span? Does the most important component of longevity lie in our genes?

2.2 SPECIFIC AIMS

The following specific aims are addressed in the 4 studies included in this thesis:

1. To investigate the impact of major age-related diseases (such as dementia, cancer, and cardiovascular diseases) on survival time. We hypothesized that type and duration of disease have different impacts on survival (Study I).
2. To explore the influence of the apolipoprotein E (*APOE*) gene on mortality in old age. We addressed this aim by testing the following hypotheses: 1) *APOE* ε2- and ε4-allele have different associations with survival; and 2) the association between allelic-variation in *APOE* and survival is probably mediated by dementia and ischemic heart and cerebrovascular disease (IHCD) (Study II).
3. To examine the associations between various modifiable factors with length of life, taking in account social background and medical conditions such as chronic diseases and multimorbidity. We hypothesize that: 1) lifestyle behaviors predict survival after age 75 and even among the oldest old (85 years and older); 2) combinations of different modifiable factors have different associations with survival (Study III).
4. To detect the combined effect of lifestyle and genetic factors on lifespan by exploring 2 hypotheses: 1) allelic-variation in genes might have a certain influence on survival and 2) longevity may result from a complex relationship between genetic and lifestyle factors (Study IV).

3 METHODS

The data in this thesis are gathered from the Kungsholmen Project.

3.1 THE KUNGSHOLMEN PROJECT

3.1.1 Study population

The Kungsholmen Project is a community-based longitudinal study on aging and dementia that was launched in 1987. The initial population included all registered inhabitants (n=2368) who were living in the Kungsholmen district of Stockholm and were aged 75 years and older.[259,260] Of these, 76.4% agree to participate in the project (n=1810). The baseline survey (1987–1989) included screening phase with a brief cognitive test (mini-mental state examination – MMSE) for all participants followed by a clinical phase for persons with cognitive impairment (MMSE≤23) and a random sample of those without impairment (MMSE>23). Five follow-ups at approximately 3-year intervals were completed before termination of data collection in 2000.

The baseline population in the Kungsholmen Project and the participants in the 4 studies included in this thesis are described in **Figure 8**.

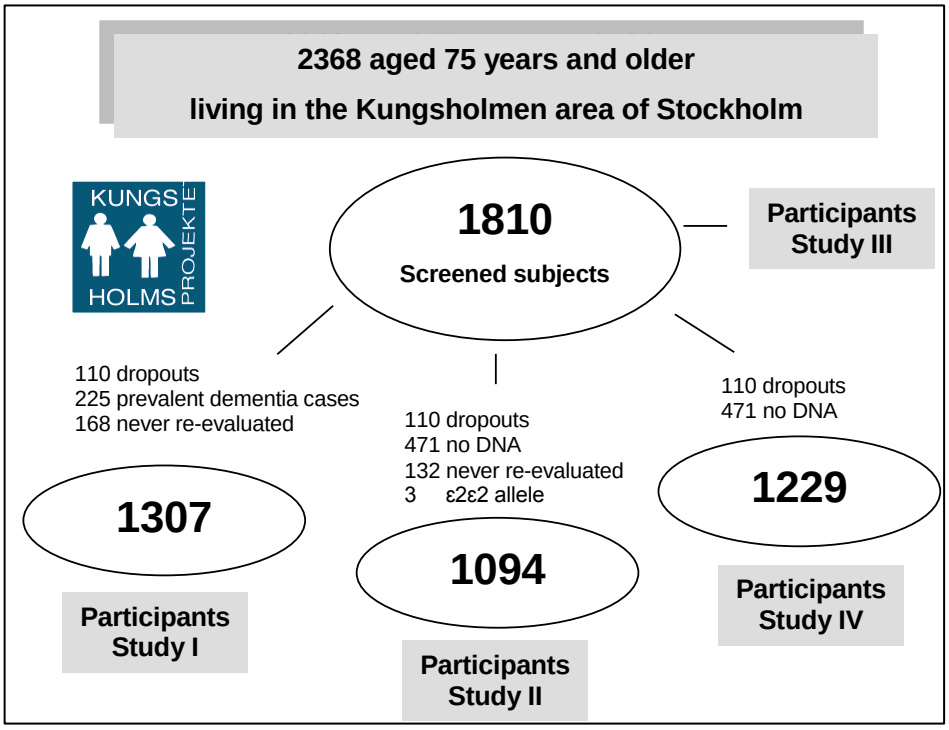


Figure 8 Kungsholmen project's baseline study population and participants in the 4 studies

3.1.2 Data collection

At each follow-up, physicians clinically examined all participants according to a standard protocol, trained personnel administered psychological tests, and nurses collected data on family and personal history.[259,260] If the parcticipant was not able to answer, an informant, usually a next-of-kin was interviewed.

Socioeconomic status (SES) assessment. SES was evaluated using 2 different variables: education and occupation. Education level was measured as total years of formal schooling. The variable was further divided into 3 main categories: primary (6 years of primary school and, in some cases, 1 year of practical vocational training); secondary (8–12 years), and university level (13+ years). All analyses were performed using the 3 educational levels; however, as results in the last 2 groups were comparable, we reported only findings related to primary versus secondary/university levels. Occupation-based SES was assessed at first follow-up by trained nurses who interviewed the participants or a relative about the lifetime work history of each participant. The interview questionnaire was developed by an expert in occupational medicine and explored lifetime work activities. Information collected about lifetime work activities included employer, job title, period of employment, and tasks for all jobs lasting at least 6 months.[261] All occupational periods were grouped according to the Swedish socioeconomic classification system (SEI) developed by Statistics Sweden (1982).[262] The longest job held during the lifetime was used to categorize people as blue-collar workers (low occupation-based SES), white-collar employees (intermediate occupation-based SES), and self-employed and academic professionals (high occupation-based SES). The last 2 groups were merged in all analyses.

Life habits assessment. Information on smoking and alcohol consumption was obtained from baseline data or, if information was missing at baseline, from data collected at the first follow-up, 3 years after baseline. Smoking history was assessed by asking participants whether they had ever smoked. Smokers and former smokers were asked how long they had smoked and the number of cigarettes smoked per day. Former smokers were also asked at what age they had stopped smoking. We categorized smoking status as current, former, and never.[263]

Data on alcohol consumption were collected using a standard questionnaire, including whether the subject drank wine, beer, or liquor, and the frequency (never or occasionally, monthly, weekly, or daily) and the quantity of alcohol drinking. The

average daily absolute alcohol intake was estimated using the frequency and quantity of consumption. Ethanol (pure alcohol) content was estimated to be 13.2 g for a bottle or can of beer [12-ounce (340.8 cc) bottle or can], 10.8 g for a standard glass of wine, and 15.1 g for a drink (counting a drink as 44 ml) of liquor.[264] The total ethanol intake for each participant was computed as the sum of contributions from beer, wine, and liquor. The total intake of ethanol was converted into standard units (1 unit = 8 g ethanol) per week for each participant on the basis of the recommendations made by the Royal College of Physicians, Psychiatrists, and General Practitioners.[265,266] All participants were then divided into 4 categories by units of ethanol intake: those who never drank; occasional drinkers (<1 unit per week), light to moderate drinkers (1–21 units per week for men, or 1–14 units per week for women), and heavy drinkers (>21 units per week for men, or >14 units per week for women).[267] At baseline only 6% of the participants reported being heavy drinkers. In our analyses, alcohol consumption was categorized as yes (light/moderate and heavy drinkers) or no (those who never drank and occasional drinkers).

BMI was considered an indicator of life habits because of its close association with food intake and physical activities. BMI was computed as the ratio of weight in kilograms to the square of height in meters (weight/(height)^2), using direct measurements, and we used widely used cut-offs to categorize the participants as overweight (BMI >25), of normal weight (20–25), or underweight (<20).[268]

A life-habit variable was created based only on smoking and BMI. Alcohol was not included because of the high percent of missing values (32%). The life-habit variable was coded as unhealthy (were overweight/underweight and were current/former smokers), moderately healthy (were overweight/underweight or were current/former smokers) and healthy (were normal-weight and had never smoked).

Leisure activity assessment. Participants were asked whether they regularly engaged in any particular activities or belonged to any organizations. If they answered yes, they were asked to specify the types of activities or organizations and to report the frequency of participation. We grouped the reported activities into mental, physical, social, and productive on the basis of the classification used in previous studies.[269] The frequency of participation in any leisure activity was initially recorded as daily, weekly, monthly, or annually. On the basis of the answers, we categorized the frequency as no participation, daily to weekly participation, and monthly participation. Owing to the statistical power of

the study, we analyzed survival in relation to participation in each type of activity (at least monthly) compared with no participation. Participants were assigned to a particular group if they participated in at least 1 of that group's activities. Mental activities included reading books or newspapers, writing, studying, doing crossword puzzles, painting, or drawing. Physical activities encompassed swimming, walking, or gymnastics. Social activities consisted of attending the theatre, concerts, or art exhibitions; traveling; playing cards or games; or participating in social groups or an organization for older people. Productive activities included gardening; housekeeping; cooking; working for pay after retirement; doing volunteer work; and sewing, knitting, crocheting, or weaving.

Social network assessment. To determine the extent of social networks, we asked participants about marital status, living arrangements, parenthood, and friendships. We also asked about frequency of contact with children and friends or relatives and how satisfied participants were with the frequency of those contacts. On the basis of their answers, we grouped the participants into the 3 social network categories: rich, moderate, and limited or poor.[270] The group with a rich social network included those who were married and lived with someone, had children with whom they were in daily to weekly contact and who found this level of contact satisfactory, and had relatives or friends with whom they were in daily to weekly contact and found this level of contact satisfactory. The group with a moderate social network included those who had any 2 of the 3 elements. The group with a limited or poor social network included those who had 1 or none of the 3 elements.

Risk profile groups. Four different risk profile groups were created on the basis of life habits, participation in leisure activities, and participants' social networks. The risk profile groups are shown in **Table 4**.

We used the participants with high risk profile as the reference group. This group included all participants who had unhealthy life habits, a limited or poor social network, and did not engage in any leisure activities. The other 3 risk profile groups were: those with a moderately high risk profile, those with a moderately low risk profile, and those with a low risk profile. The moderately high risk profile included those participants with at least 2 of the 3 risk factors. The moderately low risk profile included those with only 1 of the 3 risk factors, and the low risk profile included those who had healthy life habits, had a rich or moderate social network, and engaged in at least 1 leisure activity.

Table 4 The 4 risk profile groups include the high risk profile (dark gray), the moderately high risk profile (light grey), the moderately low risk profile (very light gray), and the low risk profile (white)

Life habits	Leisure activities	Social network	
		Moderate or rich	Limited or poor
Healthy	At least one	**LOW**	Moderately LOW
	None	Moderately LOW	Moderately HIGH
Unhealthy	At least one	Moderately LOW	Moderately HIGH
	None	Moderately HIGH	**HIGH**

Assessment of chronic diseases and multimorbidity. A disease was classified as chronic if 1 or more of the following characteristics were present: 1) the disease was permanent, 2) it was caused by non-reversible pathological alteration, 3) it required rehabilitation, or 4) it required a long period of care.[271] Chronic disorders were diagnosed by the examining physician on the basis of clinical examination, medical history, laboratory data, and current use of medications. The participants were asked by physicians to show prescription forms and/or the containers of the drugs they used. Drugs were classified on the basis of to the Anatomical Therapeutic Chemical (ATC) classification system.[272] Diagnoses of diseases were also derived from the computerized Stockholm inpatient register system for the years 1969 to 1998. The registry allowed us to identify subjects who already had the specific chronic disease at baseline (prevalent cases), as well as incident cases at follow-up. The International Classification of Diseases, Eighth and Ninth revisions (ICD-8 and 9) were used for all diagnoses in the inpatient register system, except for sensory function disorders, anemia, the various types of dementia, and major depression.

With regard to sensory function, deafness was defined the inability to hear the interviewer's voice, and visual impairment was defined as being blind or almost blind. Anemia was defined as hemoglobin<13 g/dl in men and <12 g/dl in women.[273] Dementia, various types of dementia, and major depression were diagnosed by a psychiatrist on the basis of the criteria in the Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition (DSM-III-R).[274-276]All preliminary diagnoses of dementia were reviewed by

a senior specialist, and a third opinion was solicited in case of disagreement.[259] For the persons who died before the follow-up examination, dementia was diagnosed retrospectively using information in hospital records and death certificates. Medical records and death certificates were available for all persons who died during the follow-up periods. Dementia types were classified as follows: Alzheimer's disease (AD); vascular dementia (VaD), which also included mixed dementia; and other dementia (OD), which included alcoholic dementia, dementia in Parkinson's disease, and unspecified cases of dementia. Cases diagnosed as questionable dementia have been excluded from these analyses. Age at onset of the disease was defined as the middle point between the last follow-up without the disease and the first follow-up with the disease.

Thirty-eight chronic conditions were selected, of which had a prevalence greater than 0.4% in the Kungsholmen Project study population. These 38 chronic conditions were coded as 0 (condition absent) and 1 (condition present). A summary chronic condition variable was created by counting of the number of diseases and categorizing the sum into 3 groups: no conditions, 1 condition, and 2 or more conditions. Multimorbidity was defined as the co-occurrence of 2 or more chronic conditions in the same individual, whether coincidental or not.[14]

The variable ischemic heart and cerebrovascular diseases (IHCD) was defined as the presence of the following diseases, classified according to the ICD-8 and 9: ischemic heart disease (410-414) or cerebrovascular disease (430-438). This variable included all the diseases with a documented association with *APOE*.

Genotyping procedure. A total of 1229 DNA samples from Kungsholmen Project participants were analyzed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), which differentiates genotypes on the basis of the mass of variant DNA sequences. The method is based on polymerase chain reaction (PCR), which uses an extension primer adjacent to the polymorphic site. In the PCR, allele-specific products are generated that have unique molecular mass and can be distinguished using mass spectrometry. iPLEX™ (Increased Plexing Efficiency and Flexibility for MassARRAY®) chemistry was used. The procedure was performed on the Massarray Analyzer platform from SEQUENOM®. The following genes were analyzed: angiotensin I-converting enzyme (*ACE*, rs4343 and rs1800764), apolipoprotein B (*APOB*, rs693), 3-hydroxy-3-methylglutaryl-CoA reductase (*HMG-CoA* reductase, rs3761740), methylenetetrahydrofolate reductase (*MTHFR*, rs1801133), apolipoprotein C-1 (*APOC1*,

rs4420638), lipoprotein lipase (*LPL*, rs328), lipase (*LIPC*, rs1800588), the fat mass and obesity associated gene (*FTO*, rs9939609), brain-derived neurotrophic factor (*BDNF*, rs6265), apolipoprotein E (*APOE*), insulin-degrading enzyme (*IDE*, rs1887922 and rs1544210), insulin-like growth factor 1 (*IGF1*, rs2229765), phosphatidylinositol 3-kinases (*PI3K*, rs361072), and interleukin 6 (*IL-6*, rs1800795).

Vital status assessment. Information about the vital status of the participants was derived from the official mortality data provided by Statistics Sweden. Information was available until 2003 for the Study I, 2005 for the Studies II and III, and until 2008 for Study IV.

3.2 STATISTICAL ANALYSIS

A range of statistical analyses were used in the 4 studies. To present baseline characteristics of the cohort, univariable analyses were performed with Chi-square test for categorical data and Student's t-test for continuous data. The Hardy Weinberg equilibrium in the population was tested for all genotypes.

The main outcome of interest was the time elapsed from date of birth to date of death, if it occurred during the study period. The measures used to summarize and compare the distribution of the outcome across exposure levels were mortality rates or median time to death. The regression models associated with those measures were the Cox regression for mortality rates and Laplace regression for median survival.

In multivariable Cox regression models, age was used as time-scale to better adjust for potential confounding by age.[277] The proportional hazard assumption was verified by regressing the Scaled Schoenfeld's residuals against survival time.[277,278] If any indication of no proportionality was detected, the plot of the residuals along with a smoother was studied to determine whether the mean residual varied as a function of time. If that was the case, a time-varying covariate was introduced in the model.

Empirical survival curves were produced using the Kaplan-Meier estimator of age-specific proportions of surviving individuals. A log-rank test was used to compare the statistical significance of survival curves.

We examined potential statistical interactions between covariates, including the independent variables and their cross-product terms in the same model. We formally tested for statistical interaction using the likelihood ratio test.

In Studies I and II, all diseases were modeled as time-dependent covariates to take into account the possible change over time of the diseases status (i.e. from not-affected to affected by the disease).

Laplace regression is a statistical model that makes inferences on percentiles (i.e. median) of survival time conditionally on covariates, while taking into account the presence of censored observations. In the absence of covariates, Laplace regression provides estimates of survival percentiles similar to the non-parametric Kaplan-Meier method. However, unlike Kaplan-Meier analysis, Laplace regression allows researchers to model the association between continuous exposures, adjusting for confounders, and to assess interactions in predicting survival time.[279,280]

Statistical analyses for all studies were performed and graphs created with Stata®, version 9.2 or later (StataCorp, TX, USA).

Table 5 summarizes the outcomes, exposures, potential confounders, and statistical analyses that were used in the 4 studies.

Table 5 Outcome, exposures, potential cofounders, and statistical analyses used in the studies included in this thesis

Study	Outcome	Exposures	Potential confounders	Statistical analyses	Comments
Study I	Mortality during 16 years of follow-up	Dementia, CVD, and cancer Dementia subtypes	Gender, education, prevalent cases of CVD, and cancer	Cox model, using age as time-scale	First model: sex- and education Second model: sex, education, and prevalent cases of CVD and cancer
	Mean survival time	Dementia, CVD, and cancer Dementia severity		Actual years from diagnosis to death	
	Potential years of life lost	Dementia, CVD, and cancer		Age-specific mortality was multiplied by age-specific life expectancy based on the life-table analysis	
Study II	Mortality during 18 years of follow-up	*APOE*	Gender, education, dementia, and IHCD	Cox model, using age as time-scale	First model: sex and education Second model: sex, education, and/or dementia/cancer Third model: all the previous models were reported stratifying by sex
	Years of life lost or gained	*APOE*		Comparing the mean value of age at death	
Study III	Median age at death during 18 years of follow-up	Lifestyle factors, leisure activities, and social network Combination of various patterns of modifiable factors	Age, gender, education, SES, morbidity, and multimorbidity	Laplace model	First model: age Second model: Age, gender, education, SES, morbidity, and multimorbidity Stratified by sex, age, or number of chronic conditions
Study IV	Mortality during 20 years of follow-up	Genetic factors Specific profiles of genetic and lifestyle factors	Gender	Cox model, using age as time-scale	Model: gender
	Median age at death	Specific profiles of genetic and lifestyle factors	Age and gender	Laplace model	Model: age and gender

3.2.1 Imputation of missing data

Missing values are inevitable in epidemiological studies. The problem of analyzing incompletely observed data has been extensively studied in statistical literature.[281-283]

The proportion of missing covariate data in the different studies ranged from 4% for leisure activities to 32% for alcohol consumption. The simple deletion of cases with any missing values would results in discarding up to 40% of the participants. A complete case analysis, discarding observations with incomplete information, is the standard way a statistical software deals with missing data. Concerns include loss of efficiency and possible bias when systematic differences between observed and unobserved values are related to a specific exposure-disease association.[284]

Multiple imputation techniques have been proposed as a valid alternative and are increasingly implemented in statistical software packages. We used a method of multiple multivariate imputations of missing values that has been developed for Stata, the multivariate imputation by chained equations (MICE).[283]

In the imputation model for each variable with missing values we included several predictors (i.e. age, gender, educational level, survival time, and outcome status). The specification of a proper imputation model makes more realistic the so-called "missing at random (MAR)" assumption. The assumption is that missingness is just due to chance once taking into account (conditioning on) all the predictors included in the imputation model.[285]

The results obtained from multivariable regression models estimated on the basis of 50 imputed datasets were then combined using Rubin's rule to produce overall estimates and standard errors that reflect missing-data uncertainty.[286]

4 ETHICAL CONSIDERATIONS

Informed consent was obtained at baseline from the Kungsholmen Project study participants, after explaining the aims of the project and clarifying that all information would be kept strictly confidential. If there was any indication that the participant had severe cognitive impairment, consent was obtained from a proxy, usually a next-of-kin or close relative. However, the examination or interview was interrupted if the participant expressed anguish or discomfort in any way, regardless of whether informed consent had been given by the participants themselves or by a proxy. All phases of the Kungsholmen Project were approved by the Ethics Committee at Karolinska Institutet, Stockholm, Sweden.

Studies I and II included in this thesis used the data collected from Phase I to Phase V of the Kungsholmen Project, data from medical records, and information from the Inpatient register database. Studies III and IV used the data collected during the baseline survey (Phases I & II) of the Kungsholmen Project. All 4 studies used data from death certificates.

For each phase of data collection, approval from the Ethics Committee at the Karolinska Institutet was obtained:

- Phases I & II (baseline survey): Dnr. 87:148; Dnr. 87:234
- Phase III (the first follow-up examination): Dnr. 90:251
- Phase IV(the second follow-up evaluation): Dnr. 94:122
- Phase V (the third follow-up examination): Dnr. 99:308
- Death certificate and Inpatient register data: Dnr. 99:025; Dnr. 01:020

All staff working with the Kungsholmen Project database follow the guidelines of the Swedish Council for Research in the Humanities and Social Sciences: the principles of autonomy and integrity, the rule of consent, and the demand for research.[287]

5 RESULTS

In this section of the thesis, the main results from studies I to IV are presented.

5.1 AGE-RELATED CHRONIC DISEASES AND SURVIVAL (STUDY I)

During the follow-up period (from 1987 to 1998), 958 incident cases of the following diseases were diagnosed: 738 (57%) cases of cardiovascular disease (CVD), 371 (28%) cases of dementia, and 186 (14%) cases of cancer. During a period of 16 years a total of 1140 (87%) participants died.

We investigated the association between survival over a period of 16 years and incident dementia, CVD, and cancer (**Table 6)**. The association between dementia and mortality rate was similar to the association between CVD and mortality rate. The mortality rate among those diagnosed with dementia or CVD was approximately 2 times higher than in participants without those disorders. Individuals diagnosed with cancer had a 3-fold increased mortality rate compared with those without a diagnosis of cancer.

Table 6 Adjusted hazard ratio (HR) and 95% confidence interval (CI) for the association between incident dementia, CVD, cancer and survival

	HR (95% CI)	
	Basic adjustment*	**Multivariable adjustment***
Incident dementia	1.77 (1.55 to 2.03)	1.69 (1.47 to 1.92)
Incident CVD	2.21 (1.90 to 2.57)	2.07 (1.78 to 2.41)
Incident cancer	2.92 (2.47 to 3.45)	2.71 (2.29 to 3.21)

*Basic adjustment: sex and education. Multivariable adjustment: sex, education, and baseline diagnosis of CVD and cancer.

Based on hazard ratios and fraction of incidence diseases, we estimated the reduction in the disease-specific mortality that would be observed if the population was entirely unexposed to the disease. The population attributable risks were 16% for dementia, 38% for CVD, and 20% for cancer.

We next estimated the mean survival time after the diagnosis of these diseases and the years of life lost because of premature death. Estimated years lived with incident

diagnoses of the 3 diseases were 4.1 with dementia, 4.2 with CVD, and 2.2 with cancer. The potential years of life lost were 3.4 because of dementia, 3.6 because of CVD, and 4.4 because of cancer.

The estimated years people lived with each disease or lost to it depended on age at diagnosis (**Figure 9**). In general the impact of dementia, CVD, and cancer on lifespan was higher in the youngest old group (75–84 years) than in the oldest old group (85 years and older).

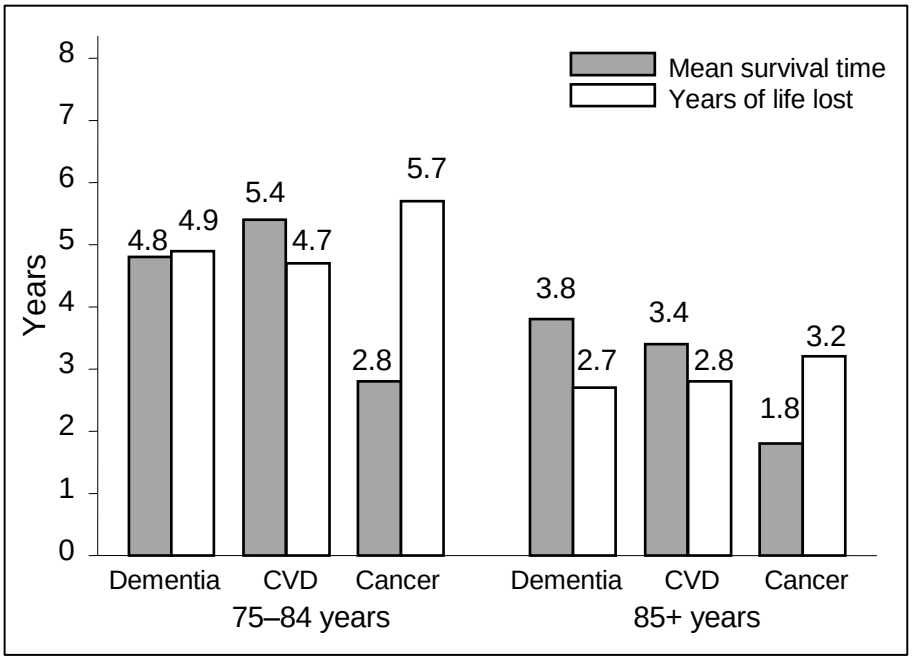


Figure 9 Mean survival time with and years of life lost because of incident dementia, CVD, and cancer stratified by age groups

The actual numbers of years lived in the different severity stage of dementia were estimated. Individuals with dementia lived, on average, for 5 months in the very mild dementia stage, 23 months in the mild stage, 14 months in the moderate stage, and 12 months in the severe stage. Women diagnosed with dementia before the age of 85 lived 1.6 years longer in the severe stage than men. Medical records were available for those who died during the first (n=318), second (n=282), and third (n=202) follow-up periods. For those people who died before the follow-up examination, the dementia diagnosis was estimated using multiple imputation technique. This sensitivity analysis provided similar results, for instance the multivariable adjusted HR were 1.81 (1.60 to 2.05) for dementia, 2.09 (1.80 to 2.43) for CVD, and 2.66 (2.25 to 3.15) for cancer.

5.2 *APOE*-RELATED MORTALITY (STUDY II)

At baseline more than half of the participants had the ε3ε3 genotype, 13% had ε2ε3 genotype, and 30% had ε4 allele. As expected, because of the effect of aging and survival selection, the proportion of participants with ε2ε3 genotype increased from baseline to the end of the follow-up time (after 18 years). This increase was followed by a concomitant decrease in the number of participants with the ε4 allele (**Figure 10**).

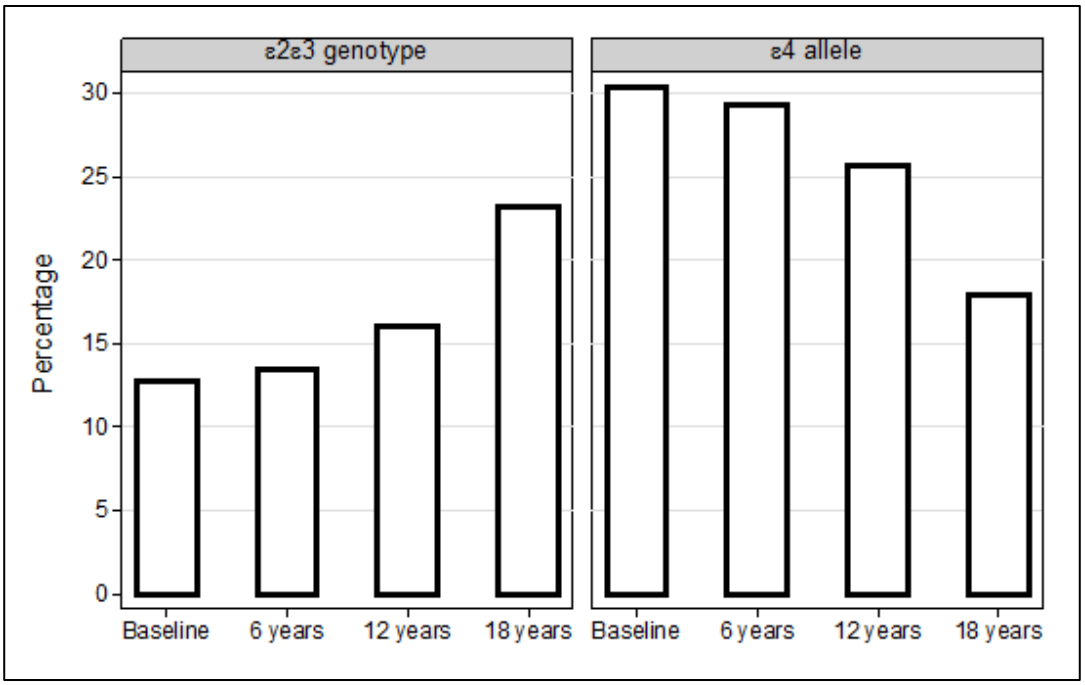


Figure 10 Percentage (%) of ε2ε3 and ε4 carriers from baseline to the end of the follow-up time

After 18 years of follow-up, only 95 (87%) participants were still alive. Survival curves for carriers of the ε2ε3 genotype and any ε4-allele were different from those of the ε3ε3 reference group (p-value<0.001). The longer survival of carriers of the ε2ε3 genotype were more pronounced among the oldest old (85 years and older). After adjustment for gender and education, ε4-carriers had a higher mortality rate (HR=1.22, 95% CI: 1.07 to 1.41) and ε2ε3-carriers a lower mortality rate (HR=0.72, 95% CI: 0.59 to 0.88), than the ε3ε3-carriers. There were so few ε2ε2-carriers in the study group that we excluded them from the analysis.

The next step was to investigate whether difference in survival might be explained by the *APOE*-related diseases (dementia and IHCD) (**Table 7**). The results in Table 7 show that the association between *APOE* and survival was modified by dementia status but not by

IHCD. Moreover, the mortality rate was mainly increased for ε4-carrier men, whereas the inverse association between ε2ε3 genotype and survival was present only among women.

APOE genotypes were missing for approximately one third of the study population. The magnitude and direction of the hazard ratios based on complete case analysis and multiple imputations were similar overall; the average of the relative differences for the sex- and education-adjusted hazard ratios was 1.8%

Table 7 Adjusted HR and 95% CI by *APOE* genotype in the whole study population and by sex

	HR (95% CI)		
	Model 1*	**Model 1 + IHCD**	**Model 1 + dementia**
Entire population			
ε3ε3	Ref.	Ref.	Ref.
ε2ε3	0.72 (0.59 to 0.88)	0.77 (0.63 to 0.94)	0.76 (0.62 to 0.92)
Any ε4	1.22 (1.07 to 1.41)	1.24 (1.08 to 1.43)	1.08 (0.94 to 1.25)
Men			
ε3ε3	Ref.	Ref.	Ref.
ε2ε3	1.07 (0.73 to 1.57)	1.17 (0.79 to 1.71)	1.11 (0.76 to 1.62)
Any ε4	1.48 (1.11 to 1.98)	1.72 (1.27 to 2.32)	1.29 (0.96 to 1.74)
Women			
ε3ε3	Ref.	Ref.	Ref.
ε2ε3	0.63 (0.50 to 0.79)	0.67 (0.53 to 0.85)	0.66 (0.52 to 0.83)
Any ε4	1.13 (0.96 to 1.33)	1.11 (0.95 to 1.31)	1.00 (0.85 to 1.18)

*Adjusted for gender and education

5.3 LIFESTYLE, SOCIAL NETWORK, AND SURVIVAL (STUDY III)

We compared the association between potentially relevant modifiable factors and median age at death. During 18 years of follow-up, 149 (8%) participants survived and 1661 (92%) did not. Overall, the median age at death was 90.0 years of age.

In the multivariable model, median age at death for women was higher than for men (difference in median age at death, years 2.4, 95% CI: 1.7 to 3.2). Participants whose weight was normal or who had never smoked lived longer than those who were underweight (-1.5, 95% CI: -2.4 to -0.6) and current smokers (-0.9, 95% CI: -1.9 to -0.0).

Participants with a high level of education (8 years or more) and those who consumed a moderate amount of alcohol survived a median of 9 months longer than those with low education (0.9, 95% CI: 0.2 to 1.7) and those who never drank (0.8, 95% CI: 0.1 to 1.6). Of all the leisure activities, physical activity was associated with the largest difference in median survival; those who were physically active survived 2 years longer than those who were physically inactive (2.0, 95% CI: 0.7 to 3.3). Further adjustment for multimorbidity attenuated the differences in median survival.

The next step in the analysis was to combine the modifiable factors in 4 different groups: low risk profile, moderately low risk profile, moderately high risk profile, and high risk profile. The median survival of individuals with a low risk profile (healthy lifestyle behaviors, participation in at least 1 leisure activity, and a rich or moderate social network) was 5.4 years longer than those with a high risk profile (unhealthy lifestyle behaviors, no participation in leisure activities, and a limited or poor social network). Even among the oldest old (85 years and older) and individuals with chronic conditions, the median age at death was 4 years higher for those with a low risk profile than those with a high risk profile (**Figure 11**).

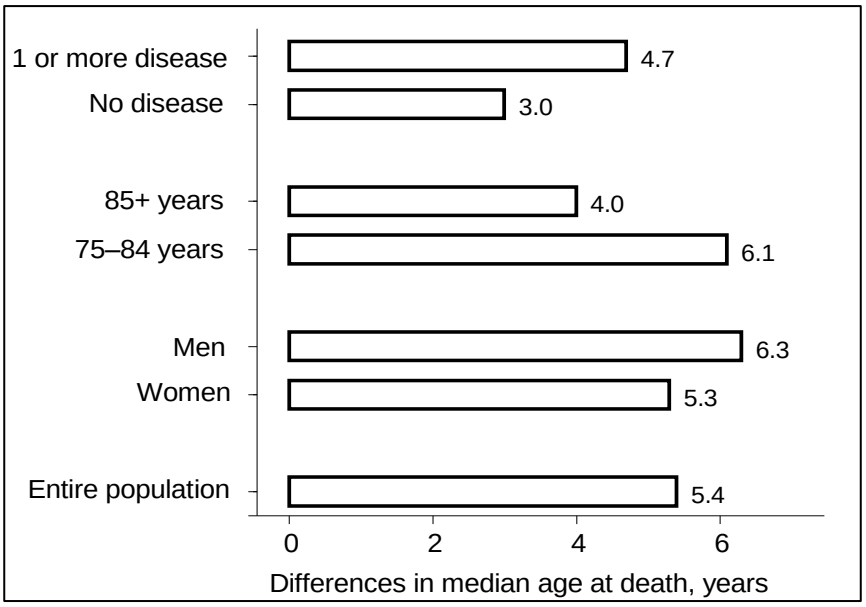


Figure 11 Differences (in years) in median age at death for the people with a low risk profile compared with the high risk profile group, in the whole population, by sex, by age, and by health status

The proportion of missing covariates data was 4% for leisure activities, 19% for BMI, 28% for smoking, and 32% for alcohol consumption. The magnitude and direction of the

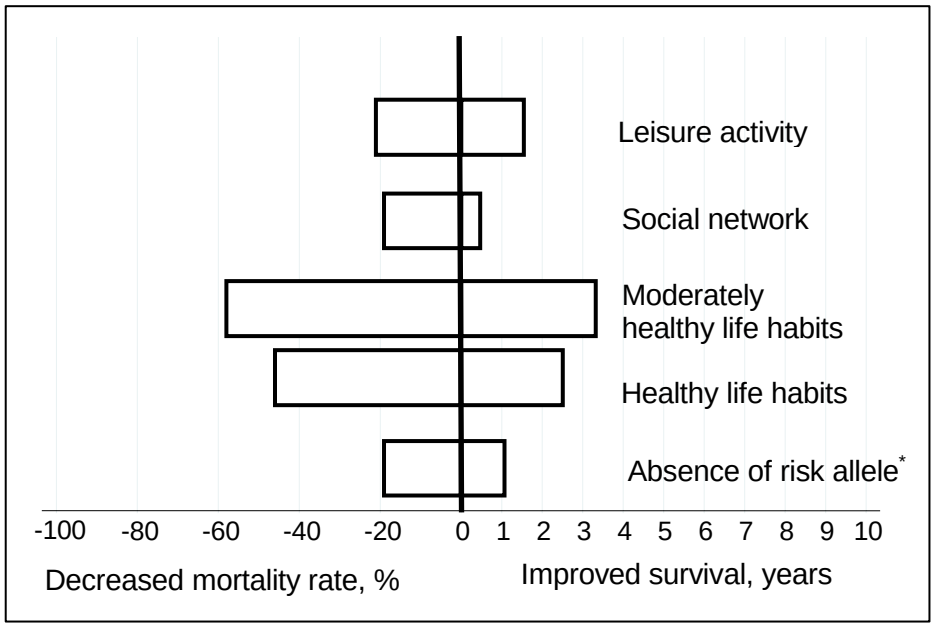
differences in median age at death based on the main analysis of complete data (60% of the cohort) and the sensitivity analysis of multiple imputation were similar.

5.4 INTERPLAY BETWEEN LIFESTYLE AND GENETIC FACTORS (STUDY IV)

We investigated variations in 14 candidate genes (16 SNPs) associated with diseases that might have an impact on lifespan or with a mechanism that might influence lifespan. During 20-years of follow-up period 1187 (97%) deaths occurred. Overall, 50% of the participants lived to be 90.6 years and older (median age at death). After controlling for age and gender, we found that increased mortality rate was associated with allelic variation in 4 genes related to cardiovascular diseases and metabolism: *APOE* ε4 compared with no ε4 allele (HR=1.26, 95% CI: 1.11 to 1.44), *APOC1* G-carriers compared with AA-carriers (HR=1.22, 95% CI: 1.08 to 1.38), *IDE* C-carriers compared with TT-carriers (HR=1.20, 95% CI: 1.06 to 1.36), and *PI3K* G-carriers compared with the AA-carriers (HR=1.12, 95% CI: 1.00 to 1.27). Gene-gene interactions were tested one by one while adjusting for the remaining genes associated with mortality. We did not find any statistical significant interaction among the genes studied.

A binary variable was created, coded as at least 1 risk allele (alleles associated with increased mortality in the analyses) versus no risk alleles. Carrying at least 1 risk allele was associated with 24% higher mortality rate than not carrying any risk allele. However, individuals with at least 1 risk allele and a healthy lifestyle had substantially lower mortality rate than those with no risk allele and an unhealthy lifestyle (HR=0.33, 95% CI: 0.18 to 0.62).

Figure 12 shows the multivariable adjusted association between genetic polymorphisms and mortality, taking lifestyle factors into account. Individuals without any of the risk alleles previously found to be associated with mortality had 20% less rate of death than carriers of at least 1 of the risk alleles, corresponding to a difference in median age at death of 1 year. Healthy life habits, participating in leisure activities, and having a moderate or rich social network were associated with a 20–60% decreased rate of death and up to a 3-year increase in lifespan.



*Among the following genes: *APOC1*, *APOE*, *IDE* (rs1887922), and *PI3K*

Figure 12 Adjusted HR and differences in median age at death (years) according to genetic and lifestyle factors

The combined absence of risk alleles and presence of healthy lifestyle was associated with 80% lower mortality rate and 6 years longer median lifespan than the combination of at least 1 risk allele and unhealthy lifestyle (**Table 8**).

Table 8 Adjusted medians age at death and 95% CI according to different genetic and lifestyle profiles

	Genetic factors	
Lifestyle profiles	**At least 1 risk allele (n=987)**	**No risk allele (n=168)**
Unhealthy	83.8 (81.6 to 86.0)	84.9 (82.6 to 87.2)
Healthy	89.2 (86.5 to 91.9)	90.3 (87.7 to 92.9)

We found similar magnitude and direction of associations between genetic and lifestyle factors in the main analysis of complete data and the sensitivity analysis of multiple imputations.

6 DISCUSSION

6.1 SUMMARY OF THE MAIN FINDINGS

This research project explored the association between survival and medical, lifestyle, and genetic factors by analyzing longitudinal data from a community-based cohort of men and women 75 years of age and older. The main results can be summarized as follows.

6.1.1 Age-related chronic diseases

- Of the 3 diseases studied, cancer was the most strongly associated with mortality when comorbidity was taken into account. A person with a cancer diagnosis had a 3-fold higher mortality rate than a person without cancer, and 4 potential years of life lost.
- The patterns of mortality associated with dementia and CVD detected were similar in terms of excess mortality rate (2 times higher than in those without the disease in question) and years of life lost (approximately 3.5 years).
- For all 3 diseases, time from diagnosis to death tended to be shorter in those who were diagnosed at older ages.
- Half the years lived with dementia were spent in the moderate and severe stages of the disease.

6.1.2 *APOE*-related mortality

- Findings suggested that *APOE* alleles played different roles in survival in elderly women than they played in elderly men. The mortality rate was 40% lower in women carrying the ε2 allele than in women with the ε3ε3 genotype. The ε4 allele was associated with 50% higher rate of death among men but not among women.
- Dementia accounted for the majority of the increased mortality rate in those with the ε4 allele, but the protection associated with the ε2 allele remained after adjustment for dementia.
- IHCD did not change the mortality rate among ε4- and ε2-carriers.

6.1.3 Lifestyle factors

- A healthy lifestyle and a rich social network were positively associated with longevity among 75 years and older, prolonging their lifespan.
- As expected, presence of a chronic disease and especially of more than 1 disease was associated with reduced lifespan. Current smokers died about 1 year earlier than non-smokers. Former smokers did not differ from those who had never smoked in terms of age at death. Participants who were underweight died 1 year earlier compared with those who weight was normal.
- Women, as expected, lived longer than men. The median difference in lifespan was 2 years. A similar increase in length of life was noted in those who reported being physically active (walking, swimming, or exercising a minimum of once a month).
- Individuals aged 75 and older who were non-smokers, participated in leisure activities at least once a month, and had good social support lived about 5 years longer than inactive smokers with poor social support.
- One additional important finding from the study was that the negative association between a chronic disease and survival is moderated by a low risk behavioral profile.

6.1.4 Lifestyle and genetic factors

- Genetic risk factors were relevant for survival after age 75. Variations in 4 different genes (*APOC1, APOE, IDE,* and *PI3K*) were associated with mortality. The higher mortality rate associated with certain alleles ranged between 12-20%.
- The results suggest that healthy lifestyle may counteract the association between carrying 1 or more risk alleles and mortality. Those who carried at least 1 risk allele had 24% higher rate of mortality than those with no such alleles. Participants with at least 1 risk allele who had a healthy lifestyle, however, had about 70% lower rate of mortality than those with no risk allele plus an unhealthy lifestyle.

- Those with no risk alleles and a healthy lifestyle had 80% decreased mortality rate and 6 years longer median lifespan than people with at least 1 risk allele and an unhealthy lifestyle.

6.2 METHODOLOGICAL ISSUES

Different sources of systematic errors or bias are likely to occur in observational studies: selection bias, information bias, and confounding. It is important to evaluate the possible role of systematic errors when interpreting observed results. A qualitative discussion of potential biases in the 4 studies follows.

6.2.1 Internal validity

6.2.1.1 Selection bias

Selection biases are distortions in the exposure-outcome association that result from procedures used to select participants and from factors associated with study participation. This type of systematic error arises when the exposure-outcome association is different in those who participated and all those who were eligible for the study, including those who did not participate.

The Kungsholmen Project is a prospective study of a community-based cohort of people 75 years and older drawn from a geographically defined area. The dropout rate in the screening phase (Phase I) of the project was 23.6% (558/2368). The main reasons for dropout included: refusal (12.4%), death (7.6%), and moving from the area (3.6%).[259] The personal characteristics of those who refused to participate and those who moved did not differ from those of the participants. However, the 181 who dropped out because they died were older than the participants and were more often men. It is likely that drop-outs led to an underestimation of the mortality rate and overestimation median age at death, especially for the oldest old men (85 years and older). In the clinical phase (Phase II) of the baseline survey, 110 subjects dropped out for reasons similar to the reasons for dropout in Phase I.

In the analysis in Studies I-IV, we used advanced statistical methods for multiple imputations to evaluate the way in which missing data might affect the observed findings. Overall, results based on complete subjects and multiple imputed datasets

were very similar, suggesting that the subsample of people included in the analysis was a random subset of the entire study population.

6.2.1.2 Information bias

Information bias occurs whenever there are systematic errors in the assessment of the main variables used in the analysis.[288]

Misclassification of the outcome

Nordic countries have a long tradition of collecting data on deaths.[289] Causes of death have been registered in Sweden since 1751 (records from 1952 and onward have been computerized). Using a unique personal identification number, the cohort was linked to the Cause of Death Register to identify deaths. This register provides nearly 100% complete case ascertainment in Sweden; therefore misclassification of the outcome (survival status) is unlikely.

Misclassification of the exposures

Misclassification of chronic diseases: dementia, CVD, and cancer. Bias in the information on incidence of chronic disease would have been an important concern if information about disease incidence were solely based on self-reported information. To minimize this type of bias, we used different sources of information on the health status of the participants: interview-based questionnaires, national registry data, and clinical examinations.

Misclassification of lifestyle factors. A structured interview by trained nurses was used to obtain information on lifestyle factors such as smoking habits, alcohol consumption, social network, and leisure activity. If a participant was not able to answer because of disease at baseline, a next-of-kin was interviewed. Previous research has shown that the validity of structured interview assessment is higher than that of self-administered questionnaires.[290] On the other hand, a possible limitation of face-to-face interviews is that some participants may report behaviors not entirely consistent with the reality. Moreover, information collected from next-of-kin might be less precise than information reported by the participants themselves.

Misclassification of genetic factors. The SNP genotyping method is based on MALDI-TOF analysis, performed on the MassARRAY Platform from SEQUENOM. This technique has the potential to provide highly accurate identification of genetic variations in DNA samples. The first step in assessing genetic variation in DNA samples is usually amplification of the DNA sequences of interest using polymerase chain reaction (PCR). This technique is based on thermal cycling and DNA replication to obtain many copies of a short fragment that harbor the variation of interest. Since this is the first step in detecting variations, optimization of its conditions is crucial. The quality of the DNA is also important for this initial step. In our cohort, we collected DNA from blood samples. Even though the blood was stored for a long time, we could efficiently amplify the DNA sequences of interest using PCR, and the desired SNPs were analysed after a number of optimization steps. Therefore it is unlikely that we have introduced any bias with the genotyping method.

In our prospective studies, all the exposures were assessed before the occurrence of death. Therefore any remaining classification errors would be independent of the future outcome. Non-differential misclassification of the exposure would lead most likely to attenuation of the observed findings.

6.2.1.3 Confounding

Confounding occurs when the observed exposure-outcome association or lack of association is distorted because of extraneous factors mixed with the actual exposure effect (which may be null). The parameters that govern the magnitude and direction of the bias are the confounder-exposure and the confounder-outcome associations. Confounding can lead to overestimation, underestimation, or even change in the direction of the apparent exposure-outcome association. Moreover, we could not fully control for bias if there are errors in the measurement of confounders. In such a case, residual confounding would persist.

The epidemiology of human longevity is a particularly challenging area of research because longevity is affected in complex ways by genetic, environmental, and social factors. In the analyses, we simultaneously took into account a variety of factors (age, gender, educational level, history of chronic diseases, lifestyles, and genetic

background). We found minimal differences between findings from models of increasing complexity. In general, after adjustment for age and gender, the addition of other possible confounding factors had minor impact on the main findings.

The strongest predictor of longevity is age, and exposures like incidence of chronic diseases and lifestyle may vary with age. As suggested in epidemiological literature, we therefore used age as the primary time scale in all models for survival analysis to better control for this predictor. Use of the Swedish personal identification number enabled us to measure the age of the participants with almost no possibility of error.

Leisure activities, social network, and life habits can have both immediate and cumulative effects. Because we assessed modifiable factors only at baseline, we could not assess the relationships between survival and changes in modifiable factors over the lifespan. Furthermore, repeated measurements of exposure would have provided a better understanding about whether accumulation of factors over the lifetime affects the associations between survival and lifestyle or social factors. It is also possible that individuals who were active at younger ages entered old age with more favorable circumstances: established activity habits, a greater likelihood of a wider social network and beneficial health behaviors, and better health in general. These circumstances form a foundation that facilitates continued participation in activities, which in turn further promotes health.

6.3 REVERSE CAUSALITY

An important issue when studying leisure activities and social network is reverse causality, which suggests that the association between lifestyle factors and health is more strongly driven by health (which leads to greater participation in activities) than by activities (which lead to health). Healthier individuals tend to participate in leisure activities to a greater extent than those who are less healthy, and they may also be more inclined to participate more regularly and with greater intensity.

Correspondingly, a socially isolated and sedentary lifestyle may affect health and well-being negatively, which in turn would create additional barriers to engaging in activities. In Study III, the count of number of chronic diseases was used as a proxy for the health status of the participants to help control for this potential confounder.

6.4 INTERPRETATION OF THE FINDINGS

The results of this project make it clear that the aging phenotype is very heterogeneous. Longevity was achieved in different ways and via different combinations of genetics, lifestyle, and health status.

6.4.1 Survival and age-related chronic diseases

Cancer and cardiovascular diseases are leading causes of death and disability among the elderly population.[103] However, when compared with other age-related diseases, dementia emerges as the strongest determinant of functional dependence.[291] In agreement with previous reports, in Study I we found that cancer had the strongest effect on mortality when comorbidity was taken into account.[292] Persons who developed cancer after age 75 had a 3-fold higher mortality rate than those without the disease and their life was shortened by 4 years. The association between dementia and mortality was similar to the association between CVD and mortality in terms of excess rate of death and years of life lost. The excess mortality associated with dementia that we found (HR=1.8) is in agreement with the results of both French[293] and American[294] studies but is slightly lower than the associations found in other community-based studies.[292,295,296] After comorbidity was taken into account, the mortality rate associated with CVD was similar to that found in a previous report.[297]

We also estimated the mean survival time after the diagnosis of dementia, CVD, and cancer. Estimated years lived after incident diagnosis was 4.15 for dementia, 4.25 for CVD, and 2.25 for cancer. The mean survival time after the onset of dementia estimated in other studies[292,293,298,299] is similar to the estimation in our study. In general, the impact of dementia, CVD, and cancer on lifespan was higher among the youngest old people (75–84 years of age) than the oldest old group (85 years and older).

The years lived after dementia onset are years of progressive disability, paralleled by an increased need for formal and informal care. In particular, women lived around 30% of this period in the most severe stage of dementia.

6.4.2 Survival and *APOE*

The majority of the previous reports that have analyzed the association between *APOE* and survival have been cross sectional in design. They have found that the frequency of the ε4 allele is lower in older age groups (octogenarians, nonagenarians, and centenarians) than in younger and middle-aged people.[185,188,189,197,209,212-220] However, the results might be dependent by of cohort effect. Eight reports have provided longitudinal data on *APOE* and mortality; their findings were inconsistent. Three studies did not find an association,[229-231] and 5 studies found a significant association.[224-228] The inconsistent results may be explained by limited sample sizes, different lengths of follow-up, and different age distributions in the samples studied.

We found that the *APOE* gene was associated with increased rate of mortality in our cohort. Mortality rate was 22% higher in ε4 allele carriers than in those with the ε3ε3 genotype, but a 28% decreased rate was observed in carriers of the ε2 allele. Adjustment for IHCD did not change the mortality rate among ε4 or ε2 carriers. Dementia accounted for the majority of the elevated mortality rate due to the ε4 allele but did not change the protective effect of the ε2 allele. Both effects were strongly modified by gender. Results suggest that men (but not women) with the ε4 allele have increased mortality rate, and that the ε2 allele has a relevant protective effect only in women. These gender differences should first be confirmed and then further explored to understand the biological mechanisms behind them.

6.4.3 Survival and lifestyle factors

Many factors can influence human longevity, but modifiable risk factors are especially relevant as they are amenable to intervention. Lifestyle, social networks, and leisure activities have been studied individually in relation to longevity in several studies, and other studies have examined the possible association of these factors with longevity while taking into account their coexistence and interactions.[81-92]

Our study confirms the negative association between smoking and survival even in old age, an association reported in some previous studies,[89,91,94] but not in others.[86,87,90] We found that smokers had a 1-year shorter median survival than those who had never smoked. The pattern of survival in former smokers in the study population was the same as the pattern of survival in those who had never smoked. Because most former smokers

in the study had quit smoking 15 to 35 years before baseline (83%), it is not clear if quitting smoking 5 to 14 years before baseline may still be associated with mortality rate in elderly people, although this seems to be suggested by our results.

The positive association between leisure activity, especially regular physical activity, and longevity found in our analysis confirms the results of some previous studies[90,91,94] but not others.[89] People who regularly engaged in physical activity lived 2 years more than sedentary people. The association between physical activity and survival was still significant after adjustment for illness that was present at baseline. We could not verify whether physical activity levels reported at baseline were important in and of themselves or were indicators of an individual's lifetime history of physical activity.

Our results on the associations between various patterns of modifiable factors and median age at death showed that compared with their respective high risk profile groups, men with a low risk profile gained more years of survival than women with a low risk profile: the women by 5 years and the men by 6 years. Even among those aged 85 years and older, the median age at death was 4 years higher if the participants had a healthy lifestyle, a rich or moderate social network, and engaged in at least 1 leisure activity. Finally, the median age at death for people with more than 1 chronic condition but who belonged to the group with the low risk profile was 87 years, 5 years later than for those with a high risk profile.

Few studies have investigated the relationship between patterns of modifiable factors and survival. In these studies, lifestyle scores were generated by pooling a variety of factors, and the results showed a strong relationship between healthy lifestyle scores and survival.[93-95]

6.4.4 Interplay between lifestyle, genetic factors, and survival

Many candidate genes have been investigated in relation to survival or longevity, especially genes already associated with diseases that shorten life or with mechanisms that might influence lifespan. However, many of the positive results have not been replicated in additional studies. We found an association between survival and allelic variation in 4 genes: 2 genes strongly related to cardiovascular diseases *(APOC1* and *APOE)* and 2 genes related to metabolism (*IDE* and *PI3K).* We did not found a significant association between any of the other genes examined and survival.

In previous studies, a high level of high-density lipoprotein (HDL) has been associated with a reduced risk of cardiovascular diseases.[300-303] This relationship persisted into old age.[304-307] Moreover, previous case-control studies showed that healthy elderly subjects have higher levels of HDL than healthy younger controls,[305] which suggests that HDL may represent a longevity factor. We selected 5 SNPs on the basis of their association with levels of HDL or LDL cholesterol reported in previous studies and examined their possible association with mortality. Variations in 2 genes were found to be associated with survival: *APOE* and *APOC1*. The *APOE* gene is the one that was most explored in previous research,[171] and findings indicated that the ε4 allele is associated with mortality. In agreement with these results, we found an increased rate of mortality among the ε4-carriers, confirming our previous results. *APOC1* was studied as candidate gene for longevity in an elderly population in Britain.[184] This study reported a difference in the frequencies of the *Hpa*I allele in elderly women and the younger women (p-value<0.05). No difference was observed between elderly men and younger men. In the present study we examined the rs4420638 SNP and found that the G-carriers had a higher mortality rate than the A-carriers. Since *APOC1* is located on chromosome 19 in the same region as the *APOE* gene, these findings may reflect potential linkage disequilibrium between *APOC1* and *APOE* genes.

In a variety of animal models from invertebrates to mammals, data indicate that aging is regulated by the insulin/IGF1 signal pathway.[308] Therefore genes that encode components of this pathway could be involved in survival. On the basis of previous research that showed an association between genetic variation in *IDE, IGF-1R,* and *PI3K* genes and longevity,[232,309-311] we investigated the role of these genes in longevity in our population. We found an increased mortality rate (20%) associated with a genetic variation in the *IDE* gene and a slight increased mortality rate (12%) was associated with any G allele of the *PI3K* gene. Mortality rate was highest for the GG genotype (20%) of the *PI3K* gene. The discrepancy in findings from different studies might be because of differences in experimental design, sample size, criteria used in selecting subjects, and different gene alleles examined. In addition, the association between polymorphisms and mortality might have a population-specific component; that is, it might be affected by the population-specific gene pool as well as by gene-environment interactions. Absence of association does not exclude the presence of a genetic impact.

Healthy lifestyle was associated with reduced rate of mortality even in those with risk alleles. Healthy lifestyle was of particular benefit to participants without any risk allele. People who carried at least 1 of the risk allele had 24% higher mortality rate than people without any risk alleles; however, if those with at least 1 risk allele had a healthy lifestyle, their mortality rate decreased. Finally, individuals without any risk allele who had a healthy lifestyle profile had approximately 80% less mortality rate, and a 6-year longer median lifespan than participants with at least 1 risk allele and an unhealthy lifestyle profile.

Future studies are needed to confirm our findings and evaluate specific gene–environment interactions in relation to longevity.

6.5 GENERALIZABILITY

No study population can ever be fully representative of all other populations. The Kungsholmen Project cohort consisted of very old individuals (75 years and older) who lived in a geographically defined central area of Stockholm. The special features of this population included a high proportion of women, high educational level, and high proportion of persons with an office-related occupation (very few farmers or industrial workers). Findings from studies carried out in this population may be generalized to urban populations of older persons in Western countries, but some caution is needed when generalizing the findings to younger persons or residents of rural areas.

7 CONCLUSIONS

I. Survival beyond age 75 is strongly associated with health status. On the whole, our findings emphasized the malignant nature of the major age-related diseases (such as dementia, cardiovascular diseases, and cancer) that increase the mortality rate and shorten life. Persons with dementia, and especially women, spent half of their life after dementia diagnosis in the severe disabling stage of the disease.

II. The association between *APOE* and survival is different for men than for women. The increased rate of mortality among ε4 carriers was present only among men and was for the most part explained by dementia. The reduced mortality rate associated with the ε2ε3 genotype, on the other hand, was present only among women and did not change after adjustment for both dementia and IHCD.

III. The associations between leisure activity, not smoking, and increased survival still existed in those aged 75 years and older; women's lives were prolonged by 5 years and men's by 6 years. These associations, although attenuated, were still present among people aged 85 and older and in those with chronic conditions. Our results suggest that encouraging favorable lifestyle behaviors even at advanced ages may enhance life expectancy, probably by reducing morbidity.

IV. Genetic risk factors were relevant to survival, but the inverse association between healthy lifestyle and mortality was still present among individuals who carried at least 1 risk allele. Participants with at least 1 risk allele and a healthy lifestyle had about 70% lower mortality rate than those with no risk allele and unhealthy lifestyle. Those with no risk alleles and a healthy lifestyle had 80% lower mortality rate and 6 years longer median lifespan than people with at least 1 risk allele and an unhealthy lifestyle.

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8 RELEVANCE AND IMPLICATIONS

A century ago, the leading causes of death were tuberculosis, diphtheria, and influenza. Thanks to antibiotics, vaccines, and improved public health measures, those top 3 killers are no longer major threats to population. Nowadays, chronic age-related diseases that gradually disable over long periods of time are emerging as the leading health challenge of the 21st century. This is quite a new development in human history. Alzheimer's and Parkinson's diseases, type II diabetes, and other diseases associated with aging could approach epidemic proportions. These diseases deprive older people of their independence, decrease the quality of longer lives, and put a burden on families and public health resources.

So far only few genetic and environmental factors have been identified as determinants of longevity, and the interplay between these factors is still unknown. Discovering the factors that play a role in longevity is an extraordinarily difficult task, but it has the potential to provide insights into central mechanisms of aging and disease.

From a clinical perspective this topic is relevant because the identification of environmental and genetic factors that affect longevity may help to better understand age-related diseases. From a public health perspective the possibility of identifying a group of individuals at risk of shorter and unhealthier lives may lead to primary and secondary preventative interventions. Identification of factors that may enhance life expectancy, probably by reducing morbidity, would have a profound impact both at the individual level and for societies by reducing care-related costs and suffering.

9 FUTURE DIRECTIONS

This project showed that there are multiple ways to survive beyond 75 years of age and that these ways work even better if different components are combined. However, questions still remain. For example, is the diet one of the keys to longevity? How much does personality change lifespan?

It would be interested to use the life-course approach to address longevity in future studies. The life-course approach considers the relevance of biological and social factors during early life, middle age, and late life to events that occur in late life, including death. This approach seeks to identify windows in time when exposures have their greatest effect on outcomes (an example is obesity at middle age, which is an important determinant of mortality in elderly people), and to determine whether accumulated exposures could have integrative or additive effects over the life course. Thus, the probability of survival after age 75 may not be determined in any single time period of the lifespan; rather, it may be a result of complex interactions between genetic susceptibility, biological factors, and environmental exposures experienced over the life course. The Swedish National Study of Aging and Care in Kungsholmen (SNACK) and the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study, which follows a middle aged or older population, offer a rare opportunity to assess the long-term relationship between midlife lifestyle and mortality in older age, for which limited data exist.

Finally, increasing life expectancy does not necessarily mean improved health in the population. Whether extra years of life gained through increased longevity are spent in good or bad health remains a crucial question to be explored.

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12 APPENDIX

List of dissertations from the Aging Research Center and the Stockholm Gerontology Research Center, 1991-2009.

1991

Herlitz Agneta: Remembering in Alzheimer's disease. Utilization of cognitive support. (Umeå University)

1992

Borell Lena: The activity life of persons with a dementia disease.

1993

Fratiglioni Laura: Epidemiology of Alzheimer´s disease. Issues of etiology and validity.

Almkvist Ove: Alzheimer´s disease and related dementia disorders: Neuropsychological identification, differentiation, and progression.

Basun Hans: Biological markers in Alzheimer´s disease. Diagnostic implications.

1994

Grafström Margareta: The experience of burden in care of elderly persons with dementia. (Karolinska Institutet and Umeå University)

Holmén Karin: Loneliness among elderly - Implications for those with cognitive impairment.

Josephsson Staffan: Everyday activities as meeting-places in dementia.

Stigsdotter-Neely Anna: Memory training in late adulthood: Issues of maintenance, transfer and individual differences.

Forsell Yvonne: Depression and dementia in the elderly.

1995

Mattiasson Anne-Cathrine: Autonomy in nursing home settings.

Grut Michaela: Clinical aspects of cognitive functioning in aging and dementia: Data from a population-based study of very old adults.

1996

Wahlin Åke: Episodic memory functioning in very old age: Individual differences and utilization of cognitive support.

Wills Philippa: Drug use in the elderly: Who? What? & Why? (Licentiate thesis)

Lipinska Terzis Beata: Memory and knowledge in mild Alzheimer's disease.

1997

Larsson Maria: Odor and source remembering in adulthood and aging: Influences of semantic activation and item richness.

Almberg Britt: Family caregivers experiences of strain in caring for a demented elderly person. (Licentiate thesis)

1998

Agüero-Eklund Hedda: Natural history of Alzheimer's disease and other dementias. Findings from a population survey.

Guo Zhenchao: Blood pressure and dementia in the very old. An epidemiologic study.

Björk Hassing Linda: Episodic memory functioning in nonagenarians. Effects of demographic factors, vitamin status, depression and dementia. (In collaboration with the Department of Psychology, University of Gothenburg, Sweden)

Hillerås Pernilla: Well-being among the very old. A survey on a sample aged 90 years and above. (Licentiate thesis)

1999

Almberg Britt: Family caregivers caring for relatives with dementia – Pre- and post-death experiences.

Robins Wahlin Tarja-Brita: Cognitive functioning in late senescence. Influences of age and health.

Zhu Li: Cerebrovascular disease and dementia. A population-based study.

2000

Hillerås Pernilla: Well-being among the very old. A survey on a sample aged 90 years and above. (In collaboration with H. M. Queen Sophia University College of Nursing, Stockholm, Sweden)

von Strauss Eva: Being old in our society: Health, functional status, and effects of research.

2001

Jansson Wallis: Family-based dementia care. Experiences from the perspective of spouses and adult children.

Kabir Nahar Zarina: The emerging elderly population in Bangladesh: Aspects of their health and social situation.

Wang Hui-Xin: The impact of lifestyles on the occurrence of dementia.

2002

Fahlander Kjell: Cognitive functioning in aging and dementia: The role of psychiatric and somatic factors.

Giron Maria Stella: The rational use of drugs in a population of very old persons.

2003

Jönsson Linus: Economic evaluation of treatments for Alzheimer's disease.

2004

Berger Anna-Karin: Old age depression: Occurrence and influence on cognitive functioning in aging and Alzheimer´s disease

Cornelius Christel: Drug use in the elderly - Risk or protection? Findings from the Kungsholmen project

Qiu Chengxuan: The relation of blood pressure to dementia in the elderly: A community-based longitudinal study

Palmer Katie: Early detection of Alzheimer's disease and dementia in the general population. Results from the Kungsholmen Project.

Larsson Kristina: According to need? Predicting use of formal and informal care in a Swedish urban elderly population. (Stockholm University)

2005

Derwinger Anna: Develop your memory strategies! Self-generated versus mnemonic strategy training in old age: Maintenance, forgetting, transfer, and age differences.

De Ronchi Diana: Education and dementing disorders. The role of schooling in dementia and cognitive impairment.

Passare Galina: Drug use and side effects in the elderly. Findings from the Kungsholmen Project.

Jones Sari: Cognitive functioning in the preclinical stages of Alzheimer's disease and vascular dementia.

Karp Anita: Psychosocial factors in relation to development of dementia in late-life: a life course approach within the Kungsholmen Project.

Nilsson Jan: Understanding health-related quality of life in old age. A cross-sectional study of elderly people in rural Bangladesh.

2006

Klarin Inga: Drug use in the elderly – are quantity and quality compatible.

Nilsson Erik: Diabetes and cognitive functioning: The role of age and comorbidity.

Ngandu Tiia: Lifestyle-related risk factors in dementia and mild cognitive impairment: A population-based study.

Erika Jonsson Laukka: Cognitive functioning during the transition from normal aging to dementia.

2007

Ferdous Tamanna: Prevalence of malnutrition and determinants of nutritional status among elderly people. A population-based study of rural Bangladesh. (Licentiate thesis)

Westerbotn Margareta: Drug use among the very old living in ordinary households-Aspects on well-being, cognitive and functional ability.

Rehnman Jenny: The role of gender in face recognition. (Stockholm University)

Nordberg Gunilla: Formal and informal care in an urban and a rural population. Who? When? What?

Beckman Gyllenstrand Anna: Medication management and patient compliance in old age.

2008

Gavazzeni Joachim: Age differences in arousal, perception of affective pictures, and emotional memory enhancement. (Stockholm University)

Marengoni Alessandra: Prevalence and impact of chronic diseases and multimorbidity in the aging population: A clinical and epidemiological approach.

Rovio Suvi: The effect of physical activity and other lifestyle factors on dementia, Alzheimer's disease and structural brain changes.

Weili Xu: Diabetes mellitus and the risk of dementia. A population-based study.

Meinow Bettina: Capturing health in the elderly population – complex health problems, mortality, and the allocation of home help services. (Stockholm University)

Agahi Neda: Leisure in late life. Patterns of participation and relationship with health.

Haider Syed Imran: Socioeconomic differences in drug use among older people. Trends, polypharmacy, quality and new drugs.

2009

Thilers Petra: The association between steroid hormones and cognitive performance in adulthood.

Rana AKM Massud: The impact of health promotion on health in old age: results from community-based studies in rural Bangladesh

Paillard-Borg Stéphanie: Leisure activities at old age and their influence on dementia development.

Livner Åsa: Prospective and retrospective memory in normal and pathological aging.

Atti Anna-Rita: The effect of somatic disorders on brain aging and dementia: Findings from population-based studies.

2010

Fors Stephan: Blood on the tracks. Life-course perspectives on health inequalities in later life.

Keller Lina: Genetics in dementia. Impact in sequence variations for families and populations.

2011

Schör Per: Gender matter. Differences and changes in disability and health among our oldest women and men.

Caracciolo Barbara: Cognitive impairment in the nondemented elderly: Occurrence, risk factors, progression.

Rieckmann Anna: Human aging, dopamine, and cognition. Molecular and functional imaging of executive functions and implicit learning.

2012

Haasum Ylva: Drug use in institutionalized and home-dwelling elderly persons.

Mangialasche Francesca: Exploring the role of vitamin E in Alzheimer's disease.An epidemiological and clinical perspective.

Lovén Johanna: Mechanism of women's own-gender bias and sex differences in memory for faces.