Determinants of Healthy Ageing: Studies of Disability and Survival among the Elderly

Determinanten van het gezonde verouderen: onderzoek naar beperkingen en overleven bij ouderen

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus

Prof.dr. H.G. Schmidt

and in accordance with the decision of the Doctorate Board.

The public defence shall be held on

Wednesday, 18th of January 2012 at 9.30 o'clock

by

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… , to those who were, those that are, and those that will be, …

Variation of *Die Lebensstufen (Strandbild, Strandszene in Wiek)* from Caspar David Friedrich (1774–1840) by Andrés Gil de Miguel

ISBN: 978-94-6169-190-3

Acknowledgements

The work described in this thesis was conducted at the department of Public Health and the department of Epidemiology of the Erasmus MC University Medical Center, Rotterdam, The Netherlands. The work was supported by NETSPAR and the NETSPAR project "Living longer in Good Health". For any grants pertaining to specific studies the reader is referred to the individual papers published in their respective journals.

The Rotterdam Study and Rotterdam Scan Study are supported by the Erasmus MC University Medical Center, the Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the Center of Medical Systems Biology, de Internationale Stichting Alzheimer Onderzoek, the Netherlands Genomics Initiative, the European Commission, and the Municipality of Rotterdam.

The contributions of the general practitioners and pharmacists of the Ommoord area to the Rotterdam Study are gratefully acknowledged.

Financial support for publication of this thesis was kindly provided by / Financiele steun voor de publicatie van dit proefschrift werd mede verzorgd door de Erasmus Universiteit en de afdelingen Maatschappelijke GezondheidsZorg (MGZ) en Epidemiologie, Rotterdam.

Contents

1. Introduction

1.1. General Introduction

The increase in life expectancy over the last century is one of society's major achievements. In particular in developed countries, though increasingly in developing countries, a higher life expectancy and a reduction in birth rates result in an ageing of the population, i.e. the shift of the median age in the population towards older ages. Thus, while human population growth approximates 1.2% annually, this population growth is not constant across all ages.[1] Annually, the population older than 60 increases by 2 – 3%, while the population older than 80 increases by 4%.[1] By 2050, more than one third of all Europeans are expected to be older than 60 years.[1] These demographic trends are supported by evidence showing that the recent increases in life expectancy in Western countries can mainly be attributed to better survival among the elderly.[2-3]

Ageing of the population poses a challenge for both developed and developing countries. Health care and pension systems are put under additional pressure because an increase in life expectancy is not necessarily desirable if it is not an increase in active and healthy life expectancy. In European countries in particular, fewer contributing inhabitants of working age support an increasing number of retirees.[4] In the Netherlands the costs of health care and welfare amounted to € 87.6 billion in 2010.[5] Expressed as percentage of gross domestic product (GDP) the costs of health care and welfare increased from 11.2% in 1998 to an estimated 14.8% of GDP in 2010. [5] Among the elderly, the growth rate of costs of care was higher.[5] It has been estimated that population ageing alone can explain an increase of about 1% in health care expenditure per year.[6] This growth rate does not include the effect of progress in medical technology developed specifically to improve the service level and health of elderly, which accounts for additional growth of 2-3% per year.[6-7] As the future development of life expectancy is uncertain, the current pension system is at risk as the present value of pension liabilities increases with higher life expectancy ("longevity risk").[8]

Health care expenditures and the pension system are both influenced by the mortality and morbidity profile of the population.[6, 9] This is because disease and illness a) determine life expectancy b) cause health care expenditures and c) influence the individual's decision to drop out of the workforce and to claim social security or pension benefits. Policy makers seeking ways to fund the increasing demands (and thus costs) for both health care and

pension systems could address both challenges by improving the morbidity profile of an ageing population.[1, 6, 8]

On the population level, there is evidence that the recent increases in life expectancy in Western countries were not accompanied by a similar increase in disability and functional limitations.[10] This phenomenon is referred to as "compression of morbidity".[11] But this success is in danger because of both the obesity epidemic and the increasing trend towards a sedentary life style. Also other evidence from observational studies among religious groups leading healthier than average life styles in terms of diet, smoking behavior, body mass index, and exercise, show impressively that morbidity can be postponed and a longer, healthier life expectancy is possible.[12-14] In fact, a recent report by the WHO regional office for Europe suggests that, for western economies in particular, measures targeting the obesity epidemic, physical exercise levels, and social involvement could alleviate the current pressures on health care and pension systems that arise from an ever growing population of elderly inhabitants.[4]

Thus, one way to improve population health is through prevention. The problem is that unlike the curative approach of modern medicine, prevention requires efforts whose benefits are not intuitively visible to the individual and to society. Monetary constraints, in conjunction with the demographic transition, might at last achieve what gerontologists did not: to divert resources away from investigating risk factors of single diseases and their treatment to research and interventions that focus on jointly postponing the onset of multiple diseases and disability.[15] This type of research promises quality of life and value (defined as health per unit of money spent) gains far higher than those associated with focusing on single diseases alone.[16-18]

Although there are several hundred "theories" of why and how human ageing occurs[1], none have successfully explained all facets of the ageing process.[19-20] Ageing is often tentatively described as the progressive loss of function, accompanied by an increased susceptibility to certain diseases such as cardiovascular disease, dementia, cancer, and ultimately death.[21-28] This approximation is not very useful for epidemiological research, and because no biomarkers of ageing have been identified,[29] there are researchers that argue that ageing as a concept should be discarded and replaced by the diseases and conditions that describe it.

Science that identifies common risk factors for age-associated conditions has the potential to allow policy makers to design interventions that positively influence population

[1] The interested reader is referred to Bengston, Gans, Putney, *et al.*, Handbook of Theories of Aging, 2nd edition, Springer, 2009.

health, reduce health care expenditure and alleviate the pressure on the pension system, simultaneously.

Studying mortality, or time to death, in population based cohorts is easier than other outcomes because it is not influenced by competing risks, as mortality is, by definition, the absorbing state in population based research. But mortality statistics only give limited information about the health of a population as individuals suffering from major health problems can survive for a considerable amount of time.[28]

Longevity, although it is often used interchangeably with life expectancy in this thesis, defines a group of people that get exceptionally old, e.g. 90 years (nonagenarians), 100 years (centenarians), or even older than 110 years (supercentenarians). Genetic studies comparing these people to the general population promise to identify genetic variants that explain survival to exceptional old age.[30-32]

Healthy ageing can be defined as surviving with highly preserved physical and mental function.[33] Disease-free survival and/or disability-free survival can be subsumed under the heading of healthy ageing. While disease-free survival can be studied by/through following individuals till the first onset of clinically manifest disease, disability is usually assessed using questionnaires. The Activities of Daily Living (ADL) scale assesses the ability to take care of oneself on a day to day basis by evaluating, among other things, ability to eat, walk, and dress.[34-35]. The Instrumental Activities of Daily Living (IADL) scale assesses more complex tasks such as managing finances, shopping, and independently taking required medications.[34, 36] These and other scales are frequently used to measure the degree of functional limitations in individuals, and to project the future health of a population.

1.2. This Thesis

The goal of this thesis is to identify risk factors for disability, disease-free survival, mortality and longevity. Specifically we asked the following research questions:

1. What genetic loci are associated with longevity and time to death and disease? (Chapter 2)
2. Do body mass index, physical activity, and happiness influence time to death and time spent with disability? (Chapter 3)
3. Which set of risk factors best predicts death and how do different groups of risk factors compare in their predictive power? (Chapter 4)

Chapter 2 investigates the association between single nucleotide polymorphisms (SNPs), longevity, and time to death and disease using genome-wide association studies. In Chapter 2.1, we investigate longevity by comparing nonagenarians, e.g. participants who survived to age 90, to participants that died before their 80th birthday. Chapter 2.2 analyzes two other related but different phenotypes: a) time to death and b) time to incident disease or death.

Chapter 3 evaluates how physical activity, body mass index, and happiness influence mortality and disability. Chapter 3.1 evaluates the potential to reduce time spent with disability by means of increasing physical activity. Chapter 3.2 similarly investigates the impact of body mass index and waist circumference on time spent with disability. Chapter 3.3 researches the obesity paradox, a phenomenon that can best be described as lower mortality in the obese in spite of a higher cardio-vascular disease risk. Finally, Chapter 3.4 describes the association between positive affect (happiness) and mortality.

Chapter 4 aims to predict mortality in the elderly population by means of a multitude of risk factors. The risk factors were grouped as follows: age and gender, socio-economic status, lifestyle, general health, prevalent disease, physiology, and genetics. A particular focus lies on the genetic component and on the comparison of the groups to each other and over different prediction intervals.

Chapter 5 is a general discussion of the research summarized in this thesis. In addition to answering the research questions described above, the methodological challenges will be described, and the answers will be put in the context of the challenges faced by the health care and pension systems described above. The discussion will conclude with policy implications and a short description of future research directions, particularly within longitudinal cohort studies.

Chapter 6 gives a short summary of this thesis.

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2. Genetic Determinants of Ageing

CHAPTER 2.1

A Meta-analysis of Four Genome-wide Association Studies of Survival to Age 90 Years or Older: The Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) Consortium

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The Journals of Gerontology: Series A. 2010; 65A(5): 478-487.

Abstract

Background: Genome wide association studies (GWAS) may yield insights into longevity.

Methods: We performed a meta-analysis of GWAS in Caucasians from four prospective cohort studies: the Age, Gene/Environment Susceptibility-Reykjavik Study**,** the Cardiovascular Health Study, the Framingham Heart Study and the Rotterdam Study, participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. Longevity was defined as survival to age 90 years and older (n=1836); the comparison group comprised cohort members who died between the ages of 55 and 80 years (n=1955). In a second discovery stage, additional genotyping was conducted in the Leiden Longevity Study cohort and the Danish 1905 cohort.

Results: There were 273 SNP associations with $p < 0.0001$, but none reached the pre-specified significance level of $5x10^{-8}$. Of the most significant SNPs, 24 were independent signals and 16 of these SNPs were successfully genotyped in the second discovery stage with one association for rs9664222, reaching $6.77\ x10^{-7}$ for the combined meta-analysis of CHARGE and the stage 2 cohorts. The SNP lies in a region near *MINPP1* (chromosome 10), a well conserved gene involved in regulation of cellular proliferation. The minor allele was associated with lower odds of survival past age 90 (OR = 0.82). Associations of interest in a homologue of the longevity assurance gene (*LASS3*) and *PAPPA2* were not strengthened in the second stage.

Conclusions: Survival studies of larger size or more extreme or specific phenotypes may support or refine these initial findings.

Introduction

Increases in longevity of the general population worldwide are an unprecedented phenomenon with significant health and social impact. Although environmental factors have led to an increase in life span, there is ample evidence that genetic factors are involved in extreme longevity both in humans (1-7) and in other organisms.(8) The protective genetic factors that lead to longevity are likely to involve fundamental processes of aging that may be different from those associated with early mortality or premature onset of age-related diseases in younger individuals. The mechanisms of aging in humans are far from understood, but available evidence suggests that several pathways-- inflammation, oxidative stress and stress responses, cellular senescence, DNA damage and repair, and the growth hormone / insulin-like growth factor / insulin (GH / IGF / INS) axis-- may play key roles.(9-12) Model organisms suggest that inhibiting the GH / IGF / INS axis, which is involved in regulating cell proliferation, cell death, wound repair, and metabolism, may promote longevity by reducing oxidative stress and slowing the rate of cell replication and the accumulation of somatic-cell DNA mutations.(13) There is also evidence for other important pathways such as the heat shock proteins and heat shock factors that are highly conserved across species and play a role in pro-longevity transcription pathways. Clinical and epidemiologic investigations, including candidate gene studies, have suggested that inflammation pathways may affect lifespan and risk of age-related conditions such as cardiovascular disease (CVD) and its risk factors.(14-19) A combination of multiple genetic variants may be required for an individual to achieve exceptional longevity, which may account in part for its rarity.

Two previous studies have used whole-genome screening to identify genetic variants associated with longevity.(20;21) In a linkage analysis, the earliest report (20) identified a locus on chromosome 4 that has not been replicated. A recent report from the Framingham Heart Study (22) identified modest associations between longevity (or age at death) and single nucleotide polymorphisms (SNPs) in or near important candidate genes including *FOXO1A, GAPDH, KL, LEPR, PON1, PSEN1, SOD2,* and *WRN,* but none of the associations achieved conventional levels of statistical significance, the sample size was modest, and the genotyping platform did not cover the genome well by current standards. The advent of genome-wide association studies (GWAS) has successfully led to the discovery of novel genetic variants that have strong evidence for replication and that are outside of traditional candidate gene regions for several common

diseases.(23-29) The detection of novel genetic variants associated with longevity holds the promise to provide important insights to biologic pathways in the aging process and thus the potential to develop innovative strategies to promote a long and healthy life.

We conducted a meta-analysis of GWAS findings for longevity within an international consortium of four longitudinal community-based cohort studies that followed adults over many years. Longevity was defined as survival to age 90 years or older and a comparison group was drawn from each cohort. Further, we identifed two independent cohorts of long-lived individuals, the Leiden Longevity Study and the Danish 1905 cohort, to evaluate initial findings for the strongest allelic associations for longevity in a second discovery stage.

Methods

CHARGE consortium

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium was convened to promote the discovery of new genomic loci involved in multiple complex traits in population-based follow-up studies using genome-wide association analysis.(30) This meta-analysis used data from the CHARGE consortium, which includes the Age, Gene/Environment Susceptibility-Reykjavik Study (31) (AGES- Reykjavik), the Cardiovascular Health Study (32) (CHS), Framingham Heart Study(33-36) (FHS), and the Rotterdam Study (37) (RS).

The AGES-Reykjavik study was funded by the National Institute on Aging and was designed to examine genetic susceptibility and environmental interactions as risk factors for disease and disability in old age. Detailed phenotyping of the cardiovascular, neurocognitive, musculoskeletal, and body composition and metabolism were conducted in 5764 men and women enrolled in 2002-06 who were sampled from the 11,549 survivors of the Reykjavik study of 30,000 men and women sampled from the 1907-35 birth cohort.(31) The CHS is a National Heart, Lung, and Blood Institute (NHLBI) contract-funded cohort study designed to evaluate risk factors for coronary heart disease (CHD) and stroke in older adults.(32) Participants (n=5201) were recruited in 1989-90, with an additional 687 minorities recruited in 1992-93. The FHS is a NHLBI contract-funded cohort study initiated in 1948 to study determinants of CVD and other major illnesses. The Original Cohort comprised 5209 men and women, aged 28-62 years at enrollment who have undergone routine biennial examinations.(33;34) In 1971, 5124 Offspring of the Original Cohort participants and Offspring spouses, aged 5 to 70 years, were enrolled into the Framingham Offspring Study. Offspring participants have been examined approximately every 4 to 8 years.(35;36) In the 1990s, DNA was obtained for genetic studies from surviving Original cohort and Offspring participants. The Rotterdam Study was planned and designed in the early 1990s as a longitudinal study investigating the incidence and progression of diseases in the elderly. From 1991 to 1995 all inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were 55 years or older, were invited to participate in this study.(38) Of 10,275 eligible individuals, 7,983 agreed to participate (78%). The participants in the CHARGE studies are Caucasian by self-report. In each CHARGE study, population structure was assessed using principal components analysis, and outliers were removed. Any remaining within-study structure was adjusted for using appropriate methods.(39) The details of each participating cohort study's

genotyping platform, imputation algorithm and quality control procedures used by each study are summarized in **Supplementary Table 1**. Each study was approved by the respective Institutional Review Board and all subjects provided consent.

Longevity and Comparison Group Definitions

In the present study, achievement of longevity was defined as reaching age 90 years or older, regardless of whether the participants were still living or had since died. Genotyped participants from these studies who died between the ages of 55 and 80 years were used as the comparison group. The comparison group was limited to deceased participants to ensure that no one in the comparison group could subsequently achieve longevity. The minimum age at death was set to match the minimum age at enrollment in the RS to promote age comparability of the comparison group across the four cohorts. The maximal age at death in the comparison group was set arbitrarily at age 80 years to include the majority of deaths, to maximize the overlap between birth cohorts, and to exclude those persons who survived far beyond average life expectancy for their respective birth cohort, that is persons who nearly reached longevity. Because of the timing of recruitment, DNA collection and death, there was only partial overlap of the birth cohorts included in the comparison groups and the group of persons achieving longevity. Only Caucasian participants were included. Across the four studies there were 1836 persons who achieved longevity (144 from AGES, 557 from CHS, 362 from FHS, and 773 from the RS) and the comparison group had 1955 participants (122, 544, 355, and 934 participants from the AGES, CHS, FHS, and RS respectively). To facilitate comparison of results across studies, we imputed to 2.5 million SNPs using the HapMap CEU genotyped samples as a reference. The effective sample size for all but one of the top SNPs was >80% of the full sample size of 3791 indicating that the SNPs that weren't directly genotyped were imputed well in most studies.

Second Discovery Stage Genotyping

Among the top 24 independent regions with the strongest associations for longevity in the 4 study meta-analysis ($p<10^{-4}$), we selected the 22 SNPs that had been tested in all 4 CHARGE cohorts in two additional Caucasian cohorts, the Leiden Longevity Study cohort and the Danish 1905 Cohort. We excluded the 2 SNPs that could not be genotyped or imputed in all 4 CHARGE

cohorts. Of the 22 SNPs selected for genotyping, 2 could not be genotyped and 4 did not pass quality control procedures, thus 16 SNPs were analyzed in the second stage.

In the *Leiden Longevity Study* (7;40), a total of 950 long-lived proband siblings (mean age 94 years, range 89 to 104 years), 1750 offspring (mean age 61 years, range 39-to 81 years) and 758 partners of offspring (mean age 60 years, range 36 to 79 years) were included. The additional genotyping of selected SNPs was undertaken in all 950 long-lived probands, and these were compared to the 744 partners of their offspring and an additional 680 blood bank donors (60% men, mean age 31 years, range 18 to 40 years). All of long-lived individuals and the comparison groups were from the Leiden area in the Netherlands and of European ancestry.

Participants in the *Danish 1905 Cohort Survey* are from the Danish 1905 birth cohort ascertained in 1998 when they were aged 92 to 93 years.(41) Of the 3,600 subjects alive from that cohort, 2,262 participants enrolled in the study. Participants underwent a home-based interview on health and lifestyle parameters, physical and cognitive tests, and collection of biological material. The current genetic study comprises a total of 1644 participants from this survey, mean age 93 years (range 92-93 years), 28% men. A comparison group included 2007 Caucasians who were twins (one twin per pair) collected from all over Denmark, with a mean age of 57 years (range 46-68 years), 45% men.

Second Discovery Stage Genotyping Methods

Genotyping of the selected SNPs was performed using an iPLEX genotyping assay developed for use with the MassARRAY platform (Sequenom Inc., San Diego, CA, USA).(42) The iPLEX genotyping assay is based on mass spectrometry and enables genotyping of 25-36 custom SNPs on a sample in a single reaction. For the purposes of quality control the system first automatically calls the genotypes and then generates cluster plots for all SNPs that are inspected individually by experienced technicians who check whether the plots show clear separation of the genotype clusters. There were 2 SNPs that did not pass quality control and 2 SNPs where no heterozygotes could not be detected, thus lack of Hardy-Weinberg equilibrium was the quality control. Negative controls were included in the genotyping procedure (8 per 384-well plate) and, importantly, 4% of samples were genotyped twice to confirm reproducibility (reproducibility was $\geq$99.7%).

Statistical Analysis

Using logistic regression each imputed and observed HapMap SNP was tested for association with the longevity outcome using an additive genetic model adjusting for sex. The mean dosage of one of the alleles (a value between 0 and 2) was the predictor for imputed SNPs. The CHS additionally adjusted for field study site in the regression model, and the FHS used generalized estimating equations (GEE) to account for familial correlations. We used the ratio of observed to expected variance in the imputed SNP genotype counts as a quality control metric for imputed SNPs.(43) This ratio, multiplied by the sample size, is an estimate of the effective sample size. In the imputation software MaCH, this ratio is called r^2, as it is an estimate of the allelic correlation between the imputed genotypes and the true genotypes for the SNP. A total of 2,287,520 SNPs that had average minor allele frequency (MAF) greater than 0.01 and were genotyped or imputed in all studies with variance ratio >0.1 were meta-analyzed. The study-specific inflation factors (λ_{GC}) were computed using the set of chi-square statistics used for the meta-analysis for each study. The inflation factor is computed as the median of all chi-square statistics divided by the expected median of the statistics (approximately 0.456) for a chi-square distribution with 1 degree of freedom. We calculated a meta-analysis odds ratio (OR) for each SNP using a fixed effects model that combined logistic regression parameters and standard errors across the studies using inverse variance weights. The meta-analysis OR represents the increase in log-odds of surviving to age $\geq$90 years versus dying between ages 55 and 80 for each additional copy of the minor allele of the SNP. SNP associations were considered to be significant on a genome-wide level at $p<5 \times 10^{-8}$. The 16 SNPs in the second discovery phase effort were analyzed in the two study samples using an additive model. The results were added to the previous meta-analysis using a fixed effects model as described above. Finally, using the top 24 results, we conducted a pathway analysis with the Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/).

Results

Table 1 provides the characteristics of the persons achieving longevity and the comparison group in each of the four CHARGE discovery cohorts at the time of DNA collection. In line with the design of the study, persons achieving longevity were 10 to 20 years older than

participants in the comparison group at baseline and were more likely to be women. Between 45% and 83% of those achieving longevity were still alive at the time that longevity status was ascertained. Among those who had died, the distributions of causes of death differed between those achieving longevity and the comparison group. While 6-12 % of those achieving longevity died of cancer, more than 30% of the comparison group had death attributed to cancer. The prevalence of diabetes and a history of ever smoking were higher in the comparison group than in persons achieving longevity. The baseline prevalence of other cardiovascular risk factor levels showed substantial overlap between the two groups.

The genomic control inflation factor lambda (λ_{GC}) for each cohort was < 1.05.(44) After meta-analysis, overall inflation of the meta-analysis p-values was minor (λ_{GC} =1.034, **Figure 1**). None of the SNP-longevity associations achieved the pre-specified level of genome-wide significance of $p<5 \times 10^{-8}$ (**Figures 1 and 2**). There were 273 SNP associations with meta-analysis $p<10^{-4}$, and, of these, 7 SNP associations had $p<10^{-5}$ (**online, Supplementary Table 2**). Under the null hypothesis that there are no associations in the genome, we would expect 0.0001*~2.3 million=~230 hits. **Table 2** shows the top 24 independent SNPs associated with longevity, along with the number of supporting SNPs (additional SNPs with LD $r^2>0.80$ and $p<10^{-4}$**).** Thus, for example, there were 19 supporting SNPs on chromosome 15 in or near the Longevity Assurance Homologue 3 (*LASS3*) gene with the strongest association (OR=0.79, $p=1.2 \times 10^{-5}$) noted for rs8029244. The study specific odds ratios for the 24 SNP associations shown in **Table 2** were in the same direction and were of similar magnitude across the four cohorts (**Figure 3 and Supplementary Table 3, online**).

Of the 24 strongest independent regions shown in **Table 2**, the 22 SNPs tested in all 4 CHARGE cohorts were selected for further evaluation, and 16 were successfully genotyped in the second stage cohorts. Only one of the 16 SNP had a smaller p-value after including the replication studies in a joint meta-analysis, with the p-value decreasing about 10 fold, from 1.61×10^{-5} to 6.77×10^{-7} and corresponding OR of 0.82. This SNP, rs9664222, is ~25kb from the *MINPP1* gene **(Figure 4)**. In the CHARGE analysis, the minor allele was associated with a lower odds of survival past age 90 (OR=0.77). The Leiden study yielded a similar effect estimate (OR=0.76, p=0.0014), while the Danish study showed a non-significant trend in the same direction (OR=0.92, p=0.19). Findings for the other SNPs were inconsistent in direction of association such that the meta-analysis p values increased with inclusion of the second stage

cohorts (**online, Supplementary Table 3**). Pathway analysis did not reveal significant findings in the top associations, though some groupings were biologically plausible.

Discussion

The CHARGE consortium collaboration allowed us to conduct a meta-analysis of GWAS for longevity in a sample of long-lived individuals and a corresponding comparison group derived from the same longitudinal, community-based cohort studies. Although none of the SNP associations for longevity in the first discovery phase achieved pre-specified level of genome-wide significance, a polymorphism associated with the *MINPP1* genes was among the strongest associations observed in our sample, with effect sizes that were similar within the four cohorts. The finding related to the *MINPP1* gene was strengthened after including two additional cohorts in a second discovery phase, but did not reach genome-wide significance. Among the top 10 associations in the initial meta-analysis, additional SNP associations of potential interest in longevity include SNPs in or near *LASS3, ACCN1*, *IL20RB* and *PAPPA2*. These SNPs are near genes that have not previously been reported to be associated with longevity in human populations but are interesting because these genes are conserved in basic biological pathways.

The *MINPP1* gene codes multiple inositol polyphosphate phosphatases which are compartmentalized to the endoplasmic reticulum lumen. *MINPP1* deficient mice have no obvious defects, though targeted deletion in vitro is associated with slowed cellular proliferation(45). There is no evidence this SNP is functional; furthermore its distance from the gene shows that it is not in strong linkage disequilibrium with SNPs in *MINPP1*.(46) However, it is well known that important regulatory elements are found outside of genes. This SNP is within 50Kb of two copy number variants. The finding of a SNP near a gene regulating proliferation is intriguing because of the higher rate of cancer death in the comparison group.

The initial finding in the *LASS3* gene region was of interest because of the historical association of its homologue with longevity in yeast.(45) The *LASS* gene family contains a group of highly conserved genes that are found in all eukaryotic species. *LASS* isoforms are mammalian homologs of the yeast longevity assurance gene 1, which encodes a protein that regulates life span.(47) The strongest association was noted for rs8029244; this SNP is in the intronic enhancer region of the *LASS3* gene. *LASS3* is a member of the ceramide synthetase family, which is important in sphingolipid metabolism, cell differentiation, cell cycling and apoptosis.(45) *LASS3* may be involved in sphingolipid synthesis or its regulation.(48)

IL20RB, interleukin 20 receptor beta IL-20, plays a role in skin inflammation and the development of hematopoietic cells (49) and is of interest because of the strong associations of inflammation with the aging process.(50) IL-20 is a pleiotropic cytokine with potent inflammatory, angiogenic, and chemoattractive characteristics and is involved in inflammatory diseases such as psoriasis, atherosclerosis, and rheumatoid arthritis.(49) The *ACCN1* gene encodes an amiloride-sensitive sodium channels with two hydrophobic transmembrane regions, and a large extracellular loop, which has many cysteine residues with conserved spacing.(51;52) The member encoded by this gene may play a role in neurotransmission. *ACCN1* was found to be associated with multiple sclerosis.(53) Pregnancy-associated plasma protein A2 (*PAPP-A2*) is a metalloproteinase regulating local insulin-like growth factor pathway action.(54) Genetic deletion extends lifespan in the mouse by 30-40%(55) and is characterized by delay in thymic involution(56) and low rates of tumor incidence.(55) Although the associations reported here did not reach the a priori specified level of significance, the findings are important to report so that they can be replicated in studies without whole genome genotyping and compared to future studies, such as in centenarian studies and family studies of longevity. Effect size estimates noted here support the likelihood that longevity is a complex process, in that there were no variants with large effects, supporting the hypothesis that there may be many genes with small effects that contribute to longevity.

The strengths of this study include the community-based prospective design and the long-term follow-up of these cohorts. In all cases, vital status was confirmed using death certificates and hospital records. Another strength was our ability to use controls that were equally well characterized and were drawn from within the same cohorts. The number of long-lived individuals reported here is very large relative to other studies in the literature, allowing greater ability to identify SNPs with small effects. The cohorts were relatively homogeneous with respect to ancestry, limited to Caucasians of European decent. Our top associations were homogeneous across cohorts. Screening for latent population substructure also supported ethnic homogeneity. Thus the findings reported are less likely to be due to population stratification.

There are important aspects of the study that need to be kept in mind when interpreting the results. The differences in causes of death in the longevous individuals vs. the comparison groups are expected, as death from cancer tends to occur earlier in life than death from heart disease or dementia. Many of the long-lived people are still alive and we do not yet know what

their ultimate cause of death will be, but it is likely that cancer will be underrepresented among persons achieving longevity. Power remains a limitation. Thus, future GWAS studies aiming to identify variants for this phenotype will have to consider small effect sizes and target a sample size larger than our nearly 2000 long-lived persons. DNA collection in cohort studies is a recent enough phenomenon that relatively few cohort members who had DNA collected have had the opportunity to survive to age 90. Continuous study of these and other similarly designed cohorts will allow us to extend this study to larger numbers and to older ages.

In our case comparison analysis, we attempted to account for birth cohort, but the overlap between birth year of the comparison group and of the long-lived participants was limited. Further follow-up of these cohorts is needed to increase our ability to examine potential birth cohort effects. The study design of the cohorts examined in the second stage was different from the initial four study CHARGE meta-analysis in that the comparison groups were derived from younger participants, living and deceased, who were not from the same cohort as the individuals achieving longevity. Certainly, there are important environmental factors that would be necessary for the fulfillment of the genetic potential for longevity. Heterogeneity in environmental exposures and gene environment interactions require further study. Finally, these results cannot be extended to populations of other ancestry.

In conclusion, this meta-analysis of GWAS data for longevity from four large cohorts and two additional cohorts has implicated several genes involved in conserved, basic mechanisms of cellular function. Analysis of more extreme survival phenotypes such as centenarians, additional follow-up to increase sample size in these cohorts for this phenotype, or evaluation of more specific phenotypes such as disease-free survival may support and refine these initial findings.

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Figure 1: Quantile-Quantile plot for the 2,287,520 SNPs in the Meta-Analysis of Survival ≥90 years.

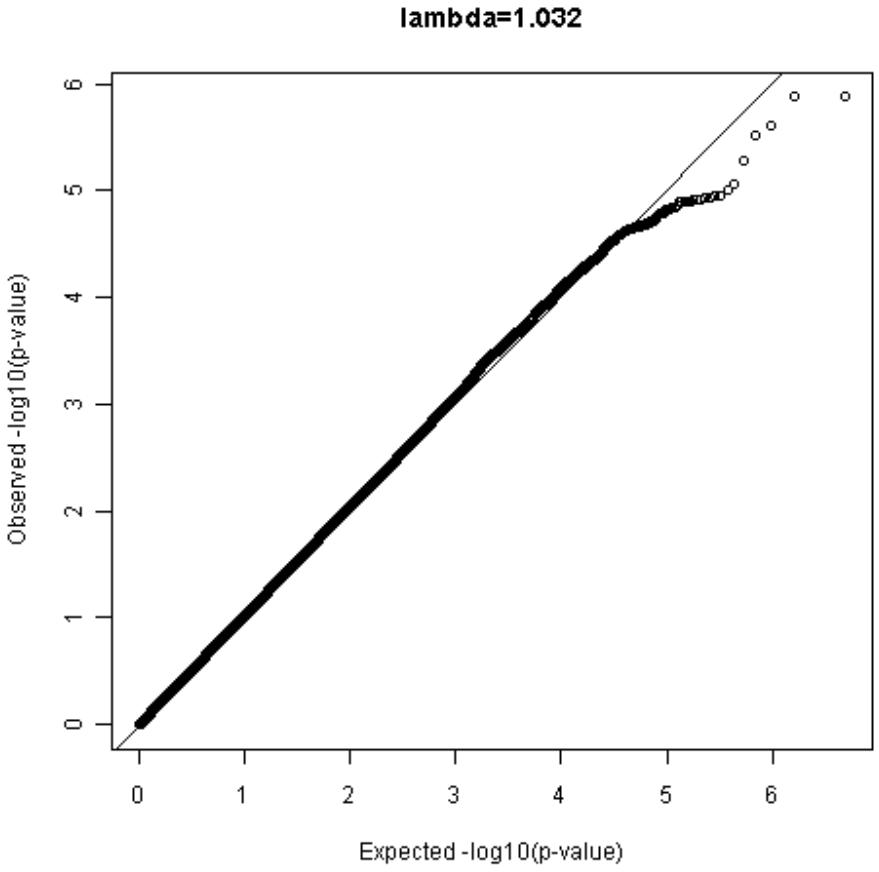

Figure 2: Plot of genome-wide association study for longevity meta-analysis (persons surviving to age $\geq$ 90, n=1836 and comparison group, n=1955) showing the -$\log_{10}$(p-values) based on the fixed-effects meta-analysis by chromosome. Line indicates threshold for genome-wide significance of 5 X 10^{-8}

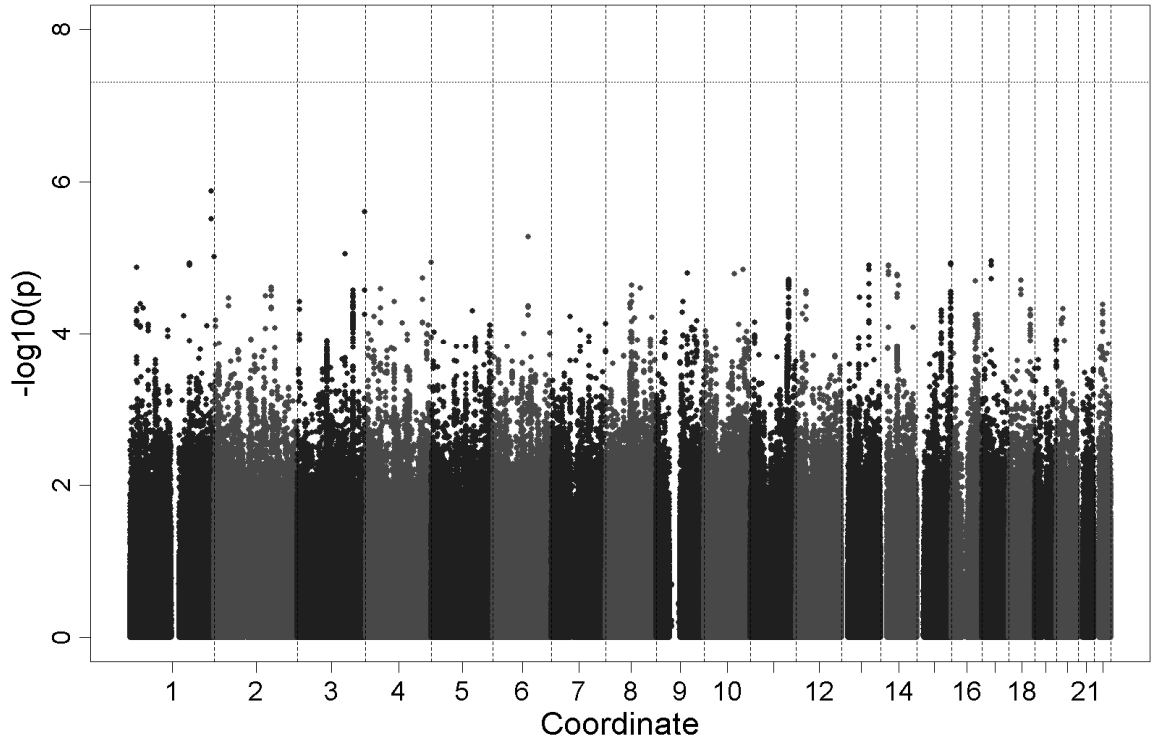

Figure 3. Study specific odds ratios and 95% Confidence Intervals for *MIPP1* (rs966422) longevity association.

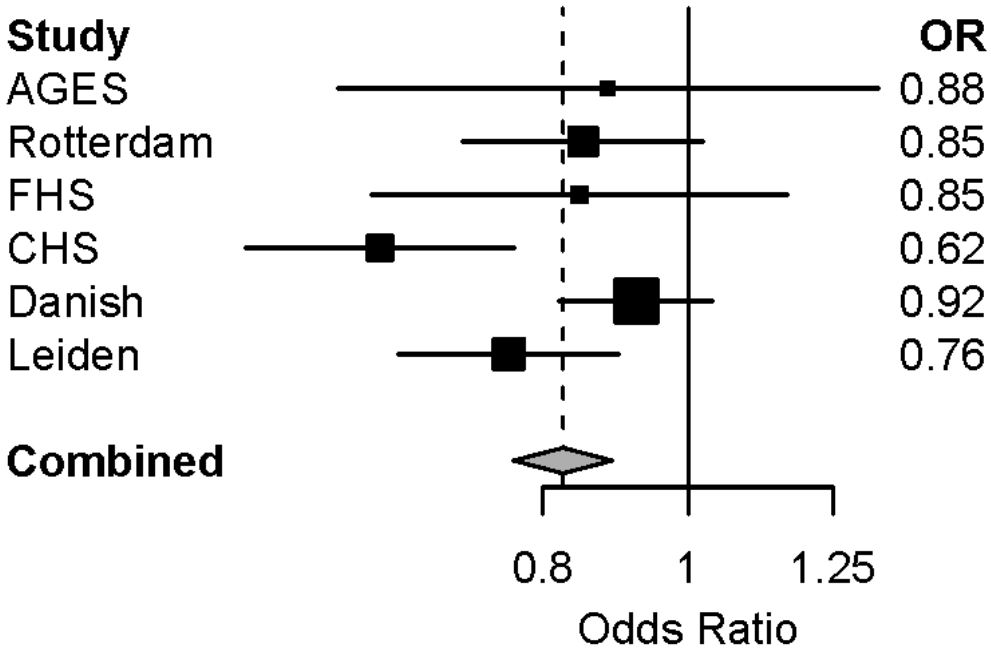

Figure 4. Regional plot for rs9664222 near *MINPP1*.

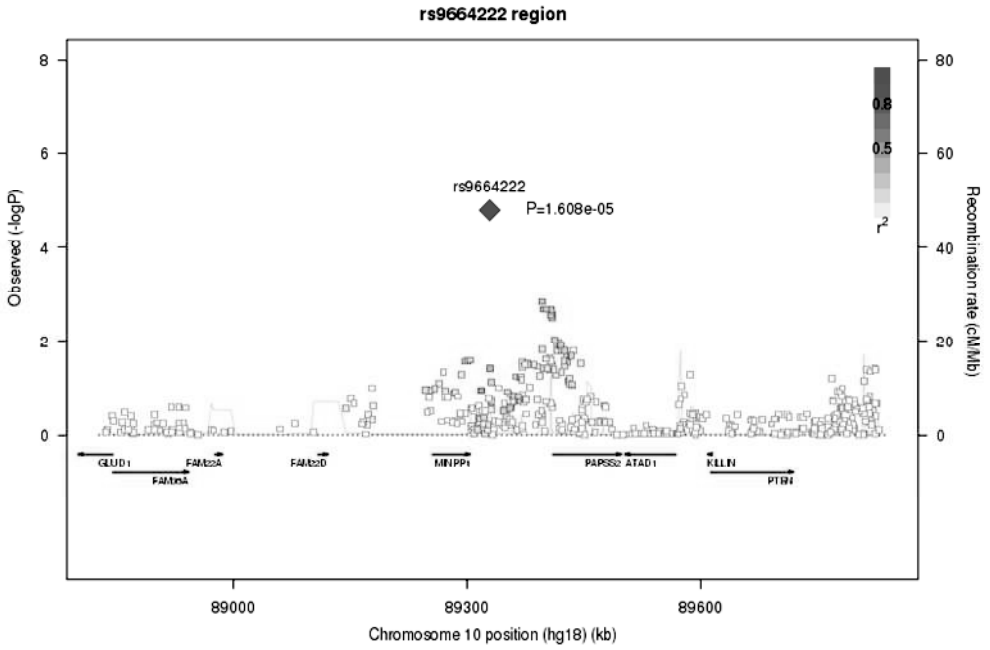

Table 1: Characteristics of Longevity Cases and Comparison Group at DNA collection

Characteristic	CHS		Framingham Heart Study		Rotterdam Study		AGES- Reykjavik	
Mean (SD) or Percent	Survival ≥90 years n=557	Comparison group n=544	Survival ≥90 years n=362	Comparison Group n=355	Survival ≥90 years n=773	Comparison Group n=934	Survival ≥90 years n=144	Comparison Group n=122
Age at DNA draw, years	79.6 (4.5)	69.5 (3.0)	87.3 (3.8)	66.5 (6.9)	83.7 (5.53)	66.5 (5.37)	88.0 (2.4)	73.8 (3.2)
Women, %	61	54	70	34	79	41	56	43
Alive, %	45	0	36	0	33	0	83	0
Cause of Death**								
CVD, %	39	33	22	23	34	32	48	39
Cancer, %	10	40	9	45	6	39	12	38
Other, %	50	27	57	25	52	27	40	23
Unknown, %	0.3	0.2	12	6	7	2	0	0
Body Mass Index, kg/m^2	25.5 (3.9)	26.6 (5.2)	26.0 (4.1)	28.0 (5.5)	26.8 (3.81)	26.3 (3.75)	25.9 (4.0)	27.4 (4.7)
Ever smoker, %	40	70	54	81.0	29	43	49.3	80
Hypertension, %	57	53	68	75	40	40	83	80
Diabetes, %	8	20	8	22	6	8	8	11
Total Cholesterol, mg/dL	210.5 (40.2)	212.2 (38.7)	198.8 (38.1)	(204.7 (47.1)	248 (49.4)	254 (46.8)	207.6 (44.3)	224.44 (42.7)

**As a proportion of all deaths for those in the Survival ≥90 group.

In the Cardiovascular Health Study, ever smoking was defined as having smoked more than 100 cigarettes or 5 packs during the subject's lifetime; hypertension was defined as a systolic ≥140 mmHg, or a diastolic ≥90 mmHg, or a history of hypertension and

taking antihypertensive medication; diabetes was defined as fasting glucose >125 mg/dl or use of insulin or oral hypoglycemic medications.

In Framingham Heart Study, ever smoking was defined as self-reported cigarette smoking of at least 1 cigarette per day for a year at any attended exam; total serum cholesterol was measured using an automated enzymatic procedure;(57) hypertension was defined as blood pressure $\geq$140/90 mmHg or on anti-hypertensive medication; diabetes was defined as fasting blood glucose >125 mg/dL, a random blood glucose of >200 mg/dL, or use of insulin or oral hypoglycemic agents.

In the Rotterdam Study, ever smoking was defined as self reported ever smoking (cigarette, cigar, or pipe); hypertension was defined as systolic blood pressure $\geq$160mmHg and/or diastolic blood pressure $\geq$100 mmHg and/or blood pressure lowering medication with an indication for hypertension, total serum cholesterol was measured using an automated enzymatic procedure,(57) diabetes was defined as self reported diabetes at baseline.

In the Age, Gene/Environment Susceptibility- Reykjavik Study, ever smoking was defined as having smoked >100 cigarettes in one's lifetime; total serum cholesterol was measured using an automated enzymatic procedure;(57) hypertension was defined as systolic $\geq$ 140 mmHg, diastolic $\geq$ 90 mmHg, use of antihypertensive medications, or self-report; diabetes was defined as fasting glucose >125 mg/dL, use of insulin or oral hypoglycemic medications, or self-report.

Abbreviations: body mass index, BMI; standard deviation,SD.

Table 2: Top 24 SNP associations ranked by p-value, for meta-analysis of 4 cohorts for survival to age ≥90 (n=1836) compared to survival to age 55 - 80 (n=1955)† and second discovery stage meta-analysis results

						Alleles								Second Discovery Stage Meta-analysis - CHARGE plus Leiden and Denmark Cohorts		
Hit No	Marker Name	Chr	Position	Closest Reference Gene	Distance (bp) from closest gene or function	Minor	Major	MAF	OR	P-value	Study Effect Direction	Number of Supporting SNPs	Effective sample size (proportion of total)	Study Effect Direction Leiden/ Denmark	OR	p-value
1	rs4443878	1	238971041	*RGS7*	34398	T	C	0.04	0.41	1.3×10^{-6}	----	10	2435	++	0.83	0.068
2	rs9825185	3	196415923	*C3orf21*	intron	C	A	0.13	1.44	2.5×10^{-6}	++++	24	0.64	--	1.10	0.045
3	rs954551	6	102886028	*GRIK2*	262554	G	A	0.25	0.77	5.3×10^{-6}	----	55	0.88	#	-	-
4	rs7624691	3	138345769	*IL20RB*	133159	C	T	0.43	0.80	8.8×10^{-6}	----	67	0.92	++	0.95	0.092
5	rs10888267	1	246126046	*OR2W3*	missense	C	T	0.45	1.25	9.7×10^{-6}	++++	2	0.92	#	-	-
6	rs9972933	17	28589381	*ACCN1*	intron	T	C	0.23	0.77	1.1×10^{-5}	----	30	0.91	+-	0.89	0.003
7	rs2739532	4	190944424			C	G	0.27	1.48	1.1×10^{-5}	?+?+	9	0.99	##	-	-
8	rs8029244	15	98826098	*LASS3*	intron	A	G	0.49	0.79	1.2×10^{-5}	----	108	0.37	+-	0.90	0.002
9	rs16850255	1	175085164	*PAPPA2*	6573	C	T	0.21	0.75	1.2×10^{-5}	----	81	0.86	++	0.92	0.041
10	rs1543505	14	22432468	*REM2*	5739	G	A	0.28	1.27	1.3×10^{-5}	++++	23	0.83	-+	1.12	0.001
11	rs7321904	13	80681190	*SPRY2*	868103	T	C	0.07	0.64	1.3×10^{-5}	----	40	0.93	++	0.92	0.179
12	rs17401847	1	20111053	*OTUD3*	UTR-3	G	A	0.15	1.38	1.4×10^{-5}	++++	26	0.90	-+	1.12	0.015
13	rs3124736	10	115487102	*CASP7*	6450	A	G	0.03	2.30	1.4×10^{-5}	+++?	0	0.89	##	-	-
14	rs690232	9	92422258	*DIRAS2*	intron	A	G	0.30	1.27	1.6×10^{-5}	+++-	68	0.64	#	-	-
15	rs9664222	10	89328613	*MINPP1*	25489	A	C	0.21	0.77	1.6×10^{-5}	----	55	0.93	--	0.82	6.77×10^{-7}
16	rs11157721	14	49586464	*LOC196913*	33655	T	C	0.39	0.79	1.7×10^{-5}	----	53	0.86	+-	0.90	0.002
17	rs4690810	4	166500130	*SC4MOL*	16456	C	T	0.35	0.79	1.9×10^{-5}	----	81	0.85	++	0.93	0.044
18	rs11605096	11	113047320	*TMPRSS5*	16162	A	C	0.12	0.71	1.9×10^{-5}	----	94	0.93	#	-	-
19	rs16972414	18	35709920	*PIK3C3*	2079276	G	A	0.30	0.79	2.0×10^{-5}	----	94	0.93	#	-	-
20	rs12935091	16	70082709	*ZNF19*	1954	G	A	0.07	0.62	2.0×10^{-5}	----	46	0.94	-+	0.80	0.002
21	rs210332	14	53262222	*BMP4*	223982	C	T	0.19	1.33	2.3×10^{-5}	++++	2	0.65	#	-	-

22	rs17369174	8	76128602	*CRISPLD1*	19256	G	T	0.10	0.69	2.3×10^{-5}	----	83	0.79	+-	0.86	0.014
23	rs6721003	2	166931758	*SCN7A*	38026	A	G	0.45	1.23	2.4×10^{-5}	++++	55	0.91	-+	1.09	0.006
24	rs4734457	8	101573533	*ANKRD46*	28644	A	C	0.10	1.75	2.5×10^{-5}	++++	0	0.99	+-	1.10	0.098

p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36.

Chr=chromosome

Odds Ratios are for each additional minor allele

Number of supporting SNPs: the number of SNPs within 500kb of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 (r^2>=0.10) and have association p-value<0.05.

† For information on all SNP associations with p-value<10^{-4} see Supplementary Table 2.

For Imputed and Direction, study-specific information is presented in the order: AGES, Rotterdam, FHS, CHS, Leiden, Denmark.

Direction: +=minor allele increases odds of survival >90, - = minor allele decreases odds of survival, ?= not tested

Genotyping requested, not completed

Genotyping not requested

Supplemental Table 1. Study specific genotyping quality control and imputation procedures

Study	AGES	CHS	FHS	RS
Array type	Illumina 370CNV	Illumina 370CNV	Affymetrix 500K and MIPS 50K combined	Version 3 Illumina Infinium II HumanHap550 SNP chip array
Genotyping center	NIA Laboratory of Neurogenetics	Cedars-Sinai Medical Center	Affymetrix Core Laboratory	Erasmus Medical Center
Genotype calling	Illumina BeadStudio	Illumina BeadStudio	BRLMM	Illumina Beadstudio
Exclusion on SNPs used for imputation	Call rate <97%, HWE $p<1^{-6}$, MAF <1%, Mishap $p<1^{-9}$, A/T and G/C SNPs, Mismatches between Illumina, dbSNP and/or HapMap position	Call rate≤97%, HWE $p<10^{-5}$, >2 duplicate errors or Mendelian inconsistencies (for reference CEPH trios) heterozygote frequency=0, not in HapMap	Call rate <97%, HWE $p<1^{-6}$, Mishap $p<1^{-9}$, Mendelian errors >100	Call rate <90%, No MAF/HWE filter
Exclusion on a per sample basis	Sex mismatch, Sample failure, Genotype mismatch with reference panel	Call rate <95%, sex mismatch, sample failure	Call rate <97%, subject heterozygosity >5 SD away from the mean, large Mendelian error rate	Call rate <97.5% sex mismatch, excess autosomal heterozygosity >0.336, outliers identified by the IBS clustering analysis
Imputation	MACH (version 1.00.16	BIMBAM10 v0.99	MACH (version 1.00.15)	Mach 1.0
Imputation Backbone (NCBI build)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)
Data handling and statistical tests	PLINK and R	PLINK, R	R packages kinship, GEE, COXPH	PLINK, ProbABLE, Mach2QTL,

Supplemental Table 2 available online at Journal of Gerontology: Medical Science.

Supplemental Table 3 available online at Journal of Gerontology: Medical Science.

Supplemental Table 2. All SNPs with p<0.0001 in the CHARGE 4-study meta analysis.

Notes

beta (OR): log odds ratio (odds ratio) of surviving past age 90 vs dying prior to age 80 for each additional copy of the minor allele.

effN: effective sample size as proportion of total sample size. Numerator: sum of total sample size x SNP imputation variance ratio or MACH r^2 for each study; denominator: total sample size (1855+1936=3791); studies with r^2<0.10 do not contribute to numerator.

minor Direction: direction of effect for minor allele for each of the 4 studies in the order AGES, Rotterdam, FHS, CHS.

min r^2, max r^2: minimum and Maximum imputation variance ratio or MACH r^2 for the 4 studies.

MarkerName	Chr	Position	Allele minor	Allele major	MAF	beta	se	p-value	minor Direction	OR	effN	min r^2	max r^2	Closest Reference Gene	Distance from Gene
rs6686814	1	20060098	T	C	0.13	0.292	0.074	7.44E-05	++++	1.34	0.97	0.90	1.00	OTUD3	21376
rs16823061	1	20063259	A	G	0.13	0.293	0.074	7.05E-05	++++	1.34	0.97	0.91	1.00	OTUD3	18215
rs7517912	1	20065463	T	A	0.13	0.294	0.074	6.64E-05	++++	1.34	0.98	0.93	1.00	OTUD3	16011
rs12064952	1	20070565	C	G	0.13	0.298	0.074	5.00E-05	++++	1.35	0.99	0.96	1.00	OTUD3	10909
rs4654910	1	20085674	A	C	0.12	0.311	0.076	4.70E-05	++++	1.36	0.97	0.91	1.00	OTUD3	4200
rs17401847	1	20111053	G	A	0.15	0.320	0.073	1.35E-05	++++	1.38	0.89	0.80	0.99	OTUD3	971
rs1033867	1	30940585	C	T	0.24	-0.263	0.064	3.97E-05	----	0.77	0.83	0.59	0.94	MATN1	17999
rs3795437	1	30979393	C	T	0.28	-0.255	0.065	8.09E-05	----	0.78	0.71	0.65	0.80	LAPTM5	1491
rs12404920	1	30979544	C	T	0.28	-0.255	0.065	7.88E-05	----	0.77	0.71	0.65	0.80	LAPTM5	1642
rs11577939	1	39809256	C	G	0.04	0.829	0.203	4.54E-05	+++?	2.29	0.43	0.04	0.70	PABPC4	5747
rs11206344	1	54625582	A	C	0.47	0.197	0.050	7.49E-05	++++	1.22	0.96	0.87	1.00	SSBP3	18985
rs6690450	1	54626667	A	C	0.49	0.190	0.049	9.09E-05	++++	1.21	0.92	0.98	1.00	SSBP3	17900
rs213490	1	54627572	A	G	0.47	0.196	0.050	8.02E-05	++++	1.22	0.96	0.88	1.00	SSBP3	16995
rs12027550	1	111623469	G	C	0.08	0.381	0.097	8.84E-05	++++	1.46	0.87	0.55	1.00	CHIA	11537
rs9804152	1	156785563	T	C	0.25	-0.268	0.067	5.74E-05	?---	0.76	0.73	0.00	1.00	OR6Y1	1044
rs16850255	1	175085164	C	T	0.21	-0.290	0.066	1.18E-05	----	0.75	0.83	0.44	1.00	PAPPA2	6573
rs12094387	1	175086498	C	T	0.21	-0.290	0.066	1.23E-05	----	0.75	0.83	0.44	1.00	PAPPA2	7907
rs1534957	1	175087680	A	G	0.21	-0.289	0.066	1.26E-05	----	0.75	0.83	0.44	1.00	PAPPA2	9089
rs3219142	1	224618691	A	G	0.19	0.318	0.081	7.86E-05	++++	1.37	0.58	0.17	0.94	PARP1	3677
rs7531849	1	238961206	C	A	0.04	-0.799	0.171	3.08E-06	----	0.45	0.64	0.26	0.97	RGS7	44233
rs4443878	1	238971041	T	C	0.04	-0.894	0.185	1.33E-06	----	0.41	0.64	0.31	0.92	RGS7	34398
rs4611001	1	238971251	G	A	0.04	-0.894	0.185	1.33E-06	----	0.41	0.64	0.31	0.92	RGS7	34188

rs10888267	1	246126046	C	T	0.45	0.220	0.050	9.66E-06	++++	1.25	0.91	0.96	1.00	OR2W3	408
rs4595985	2	3559389	T	C	0.34	0.215	0.054	6.62E-05	++++	1.24	0.91	0.81	0.97	RNASEH1	11178
rs10153531	2	3567678	A	G	0.34	0.209	0.053	8.05E-05	++++	1.23	0.94	0.82	0.98	RNASEH1	2889
rs17029781	2	42620548	C	T	0.04	0.566	0.138	4.25E-05	++++	1.76	0.73	0.15	1.02	MTA3	28626
rs17679678	2	42713218	A	G	0.04	0.703	0.170	3.40E-05	+++?	2.02	0.71	0.09	0.98	MTA3	64044
rs7584841	2	149805714	A	G	0.33	-0.210	0.053	8.46E-05	----	0.81	0.91	0.96	1.00	LOC130576	25696
rs1420356	2	149897178	G	A	0.48	-0.239	0.058	3.18E-05	----	0.79	0.73	0.55	0.84	LYPD6	1820
rs6432908	2	166926110	G	A	0.46	0.207	0.049	2.63E-05	++++	1.23	0.99	0.98	1.01	SCN7A	43674
rs1540874	2	166928270	G	A	0.46	0.207	0.049	2.64E-05	++++	1.23	1.00	0.99	1.01	SCN7A	41514
rs7590179	2	166929114	G	T	0.46	0.207	0.049	2.62E-05	++++	1.23	1.00	0.99	1.01	SCN7A	40670
rs6721003	2	166931758	A	G	0.45	0.208	0.049	2.44E-05	++++	1.23	0.99	0.98	1.01	SCN7A	38026
rs4384809	2	166932764	G	T	0.47	0.204	0.049	3.13E-05	++++	1.23	0.93	1.00	1.02	SCN7A	37020
rs10179640	2	166949233	A	G	0.45	0.209	0.051	4.49E-05	++++	1.23	0.93	0.76	1.00	SCN7A	20551
rs6710634	2	166951683	G	A	0.45	0.209	0.051	4.64E-05	++++	1.23	0.93	0.76	1.01	SCN7A	18101
rs1348807	2	176714003	C	T	0.28	-0.227	0.058	8.24E-05	----	0.80	0.91	0.80	0.96	HOXD8	9031
rs4485702	3	5364179	T	C	0.12	-0.334	0.082	4.72E-05	----	0.72	0.88	0.77	0.96	EDEM1	127537
rs4367043	3	5365506	T	A	0.12	-0.334	0.082	4.71E-05	----	0.72	0.88	0.77	0.96	EDEM1	128864
rs10510303	3	5385409	T	C	0.10	-0.372	0.090	3.72E-05	----	0.69	0.84	0.83	0.88	EDEM1	148767
rs7624691	3	138345769	C	T	0.43	-0.222	0.050	8.83E-06	----	0.80	0.92	0.97	1.03	IL20RB	133159
rs16832246	3	162592610	A	G	0.37	0.202	0.051	6.64E-05	++++	1.22	0.96	0.90	1.00	C3orf57	20045
rs11713185	3	162594051	G	C	0.37	0.202	0.051	6.47E-05	++++	1.22	0.96	0.91	1.00	C3orf57	21486
rs16832279	3	162603177	T	C	0.37	0.201	0.051	7.25E-05	++++	1.22	0.97	0.92	1.00	C3orf57	30612
rs11710086	3	162608552	T	A	0.36	0.201	0.051	6.87E-05	++++	1.22	0.97	0.93	1.00	C3orf57	35987
rs11710916	3	162612764	C	T	0.36	0.201	0.050	6.88E-05	++++	1.22	0.98	0.95	1.00	C3orf57	40199
rs1811483	3	162613594	G	A	0.36	0.203	0.050	5.35E-05	++++	1.23	0.91	0.96	1.00	C3orf57	41029
rs4287919	3	162708879	T	C	0.40	-0.202	0.050	5.93E-05	----	0.82	0.99	0.97	1.00	LOC131149	4455
rs9883089	3	162712090	A	G	0.41	-0.199	0.051	8.43E-05	----	0.82	0.97	0.94	1.00	LOC131149	7666
rs9831924	3	162721550	A	G	0.40	-0.204	0.051	5.73E-05	----	0.82	0.97	0.95	0.99	LOC131149	17126
rs4856642	3	162731020	A	T	0.40	-0.198	0.050	7.60E-05	----	0.82	0.99	0.99	1.00	LOC131149	26596
rs9831040	3	162734475	G	A	0.41	-0.198	0.050	6.93E-05	----	0.82	1.00	0.99	1.03	LOC131149	30051
rs2221517	3	162742975	T	C	0.40	-0.196	0.049	7.19E-05	----	0.82	0.93	0.99	1.08	LOC131149	38551
rs2221516	3	162746633	C	T	0.40	-0.194	0.049	8.34E-05	----	0.82	1.00	0.99	1.07	LOC131149	42209
rs7621711	3	162749821	A	G	0.40	-0.194	0.049	8.69E-05	----	0.82	0.93	0.98	1.08	LOC131149	45397
rs6781716	3	162751305	A	G	0.40	-0.199	0.050	7.62E-05	----	0.82	0.99	0.98	1.00	LOC131149	46881
rs9868834	3	162755468	C	T	0.39	-0.200	0.051	7.73E-05	----	0.82	0.98	0.95	1.00	LOC131149	51044
rs6793693	3	162772861	G	A	0.40	-0.212	0.051	3.28E-05	----	0.81	0.96	0.89	1.00	LOC131149	68437

rs7620901	3	162790010	T	C	0.40	-0.214	0.051	2.97E-05	----	0.81	0.96	0.87	1.00	LOC131149	85586
rs6441391	3	162821725	G	T	0.42	-0.200	0.050	5.82E-05	----	0.82	0.99	0.99	1.00	LOC131149	117301
rs2048801	3	162822630	A	C	0.41	-0.197	0.050	9.72E-05	----	0.82	0.98	0.94	1.00	LOC131149	118206
rs1588907	3	162825658	A	G	0.41	-0.199	0.051	7.88E-05	----	0.82	0.98	0.94	1.00	LOC131149	121234
rs9290087	3	162826610	G	C	0.41	-0.199	0.051	7.91E-05	----	0.82	0.98	0.94	1.00	LOC131149	122186
rs1399904	3	162830565	A	G	0.41	-0.204	0.051	6.72E-05	----	0.82	0.95	0.86	1.00	LOC131149	126141
rs9813121	3	162831245	G	A	0.41	-0.204	0.051	6.65E-05	----	0.82	0.95	0.85	1.00	LOC131149	126821
rs1399910	3	162832067	C	A	0.41	-0.206	0.052	6.34E-05	----	0.81	0.94	0.81	1.00	LOC131149	127643
rs1878064	3	162832324	C	T	0.41	-0.207	0.052	6.34E-05	----	0.81	0.94	0.80	1.00	LOC131149	127900
rs1399906	3	162833920	T	C	0.41	-0.209	0.052	6.05E-05	----	0.81	0.93	0.77	1.00	LOC131149	129496
rs9871565	3	162834899	T	C	0.41	-0.209	0.052	5.84E-05	----	0.81	0.92	0.76	1.00	LOC131149	130475
rs9876608	3	162835553	A	G	0.40	-0.220	0.054	4.54E-05	----	0.80	0.86	0.67	0.95	LOC131149	131129
rs9876363	3	162835601	G	C	0.41	-0.209	0.052	5.62E-05	----	0.81	0.93	0.75	1.00	LOC131149	131177
rs9877938	3	162836338	A	G	0.41	-0.209	0.052	5.51E-05	----	0.81	0.93	0.75	1.00	LOC131149	131914
rs1515741	3	162837901	C	A	0.41	-0.215	0.052	4.05E-05	----	0.81	0.91	0.71	1.00	LOC131149	133477
rs1850000	3	162839738	T	C	0.41	-0.214	0.052	4.40E-05	----	0.81	0.91	0.71	1.00	LOC131149	135314
rs1399916	3	162842069	T	C	0.41	-0.214	0.052	4.34E-05	----	0.81	0.91	0.72	1.00	LOC131149	137645
rs1515743	3	162843501	C	T	0.41	-0.215	0.052	4.25E-05	----	0.81	0.91	0.71	1.00	LOC131149	139077
rs1568020	3	162847013	T	A	0.41	-0.216	0.053	3.87E-05	----	0.81	0.91	0.70	1.00	LOC131149	142589
rs4305435	3	162849335	G	T	0.41	-0.217	0.053	3.80E-05	----	0.81	0.90	0.69	1.00	LOC131149	144911
rs2203490	3	162850383	T	C	0.41	-0.217	0.053	3.77E-05	----	0.80	0.90	0.69	1.00	LOC131149	145959
rs1515724	3	162852681	C	T	0.41	-0.220	0.053	3.18E-05	----	0.80	0.90	0.69	0.99	LOC131149	148257
rs1399915	3	162858044	T	C	0.41	-0.222	0.053	2.65E-05	----	0.80	0.90	0.69	0.99	LOC131149	153620
rs2131879	3	196320205	G	A	0.11	0.343	0.082	2.67E-05	++++	1.41	0.92	0.77	0.99	C3orf21	49902
rs2410845	3	196335311	A	C	0.11	0.357	0.089	5.54E-05	++++	1.43	0.79	0.47	0.97	C3orf21	65008
rs9825185	3	196415923	C	A	0.13	0.364	0.077	2.48E-06	++++	1.44	0.88	0.80	1.00	C3orf21	57243
rs7654633	4	6664506	G	A	0.21	-0.245	0.063	9.98E-05	+---	0.78	0.90	0.74	0.98	MAN2B2	10583
rs2970848	4	23426125	G	A	0.30	-0.209	0.052	5.96E-05	----	0.81	0.93	1.00	1.03	PPARGC1A	23383
rs12501388	4	42368069	A	G	0.08	0.426	0.107	7.08E-05	++++	1.53	0.80	0.39	1.00	ATP8A1	14190
rs12499249	4	42369790	T	A	0.07	0.425	0.107	7.13E-05	++++	1.53	0.80	0.40	1.00	ATP8A1	15911
rs17593986	4	42381287	A	G	0.07	0.421	0.103	4.54E-05	++++	1.52	0.88	0.59	1.00	ATP8A1	27408
rs4279252	4	42437007	C	T	0.08	0.477	0.113	2.53E-05	++++	1.61	0.68	0.16	0.95	ATP8A1	83128
rs2035912	4	83226499	C	T	0.08	0.377	0.092	3.79E-05	++++	1.46	0.89	0.79	1.00	HNRPD	266991
rs17293880	4	107773907	A	C	0.13	0.352	0.089	7.14E-05	++++	1.42	0.68	0.32	0.83	SCYE1	284887
rs1866218	4	166476262	G	C	0.33	-0.234	0.059	7.24E-05	----	0.79	0.82	0.55	1.02	SC4MOL	7412
rs2322292	4	166477883	T	C	0.33	-0.234	0.059	7.13E-05	----	0.79	0.82	0.55	1.02	SC4MOL	5791

rs4691183	4	166486177	T	C	0.33	-0.223	0.056	7.13E-05	----	0.80	0.89	0.66	1.01	SC4MOL	2503
rs7667236	4	166486569	A	G	0.33	-0.223	0.056	7.05E-05	----	0.80	0.89	0.66	1.01	SC4MOL	2895
rs7655392	4	166499952	C	A	0.36	-0.227	0.055	3.52E-05	----	0.80	0.90	0.76	0.96	SC4MOL	16278
rs4690810	4	166500130	C	T	0.35	-0.234	0.055	1.86E-05	----	0.79	0.93	0.82	0.97	SC4MOL	16456
rs2333491	4	177795858	G	A	0.34	0.211	0.054	9.87E-05	++++	1.23	0.96	0.91	0.99	VEGFC	45826
rs1872500	4	177796115	G	A	0.34	0.211	0.054	9.95E-05	++++	1.23	0.96	0.91	0.99	VEGFC	45569
rs6850916	4	182133382	T	C	0.30	0.270	0.068	7.69E-05	++++	1.31	0.61	0.45	0.75	ODZ3	1348748
rs2739532	4	190944424	C	G	0.27	0.395	0.090	1.14E-05	?+?+	1.48	0.37	NA	NA		NA
rs16879258	5	7918891	A	C	0.13	0.317	0.081	9.51E-05	++++	1.37	0.75	0.24	1.03	FASTKD3	3224
rs10036031	5	119731281	A	C	0.05	0.580	0.143	4.97E-05	++++	1.79	0.70	0.35	0.87	PRR16	96636
rs2569208	5	171732988	C	T	0.36	-0.198	0.051	9.04E-05	----	0.82	0.93	1.00	1.03	SH3PXD2B	39879
rs2247647	5	171749649	T	C	0.38	-0.201	0.051	7.67E-05	----	0.82	0.97	0.91	1.00	SH3PXD2B	56540
rs12202794	6	89032551	G	C	0.08	-0.480	0.123	9.91E-05	+--?	0.62	0.58	0.05	0.89	CNR1	120776
rs954551	6	102886028	G	A	0.25	-0.260	0.057	5.33E-06	----	0.77	0.92	0.93	1.04	GRIK2	262554
rs9377361	6	102972083	G	A	0.24	-0.234	0.058	5.61E-05	----	0.79	0.93	0.99	1.03	GRIK2	348609
rs9377363	6	102975329	T	C	0.24	-0.238	0.058	4.23E-05	----	0.79	1.00	0.99	1.02	GRIK2	351855
rs7771560	6	102976780	C	T	0.24	-0.238	0.058	4.50E-05	----	0.79	1.00	0.98	1.02	GRIK2	353306
rs9285551	6	102979782	T	C	0.24	-0.238	0.058	4.45E-05	----	0.79	1.00	0.98	1.02	GRIK2	356308
rs1416280	6	102993336	G	C	0.24	-0.239	0.059	4.39E-05	----	0.79	0.99	0.97	1.02	GRIK2	369862
rs12198299	6	167004265	C	T	0.36	0.207	0.053	9.77E-05	++++	1.23	0.89	0.67	0.97	RPS6KA2	43549
rs13235506	7	53753195	G	T	0.06	-0.483	0.120	5.92E-05	----	0.62	0.83	0.43	1.00	DKFZp564N2	681084
rs10252600	7	85015874	C	T	0.45	-0.194	0.050	8.92E-05	----	0.82	0.97	0.93	0.98	SEMA3D	426691
rs12698138	7	157578364	G	A	0.12	0.321	0.081	7.27E-05	++++	1.38	0.81	0.58	1.00	PTPRN2	494815
rs6472566	8	72163038	G	A	0.37	-0.204	0.050	4.08E-05	----	0.82	0.99	0.99	0.99	EYA1	109185
rs10957534	8	72170857	C	G	0.37	-0.202	0.050	4.55E-05	----	0.82	0.99	0.99	1.00	EYA1	101366
rs13277069	8	72172085	T	C	0.37	-0.203	0.050	4.07E-05	----	0.82	0.99	0.98	1.00	EYA1	100138
rs6990397	8	72174326	C	T	0.37	-0.204	0.050	3.96E-05	----	0.82	0.99	0.98	1.00	EYA1	97897
rs17799174	8	73052557	T	A	0.13	0.309	0.079	9.20E-05	++++	1.36	0.82	0.58	1.00	TRPA1	43483
rs10957743	8	76043997	C	T	0.31	-0.207	0.053	9.38E-05	----	0.81	0.95	0.89	0.99	CRISPLD1	15533
rs2956605	8	76045609	A	C	0.39	0.210	0.053	6.80E-05	++++	1.23	0.90	0.79	0.98	CRISPLD1	13921
rs11778674	8	76126543	A	C	0.10	-0.345	0.083	3.13E-05	----	0.71	0.97	0.90	1.01	CRISPLD1	17197
rs17369174	8	76128602	G	T	0.10	-0.367	0.087	2.30E-05	----	0.69	0.91	0.69	1.01	CRISPLD1	19256
rs17369209	8	76128813	C	A	0.10	-0.368	0.087	2.30E-05	----	0.69	0.91	0.69	1.01	CRISPLD1	19467
rs11785349	8	76129854	G	T	0.10	-0.368	0.087	2.30E-05	----	0.69	0.91	0.69	1.01	CRISPLD1	20508
rs17297422	8	76153367	C	A	0.10	-0.356	0.086	3.74E-05	----	0.70	0.90	0.67	1.00	CRISPLD1	44021
rs7813189	8	84285307	T	C	0.27	-0.285	0.071	6.14E-05	----	0.75	0.60	0.13	0.87	RALYL	972700

rs4734457	8	101573533	A	C	0.10	0.562	0.133	2.47E-05	++++	1.75	0.36	0.11	0.61	ANKRD46	28644
rs4348534	8	135341451	C	T	0.47	-0.200	0.050	6.05E-05	----	0.82	0.87	0.68	1.05	ZFAT1	217762
rs12003047	9	25517621	C	T	0.19	0.271	0.069	9.38E-05	++++	1.31	0.82	0.40	0.99	TUSC1	148772
rs4745348	9	76427797	T	C	0.02	1.320	0.326	5.15E-05	-++?	3.74	0.38	0.03	0.56	RORB	64138
rs13288161	9	79426258	T	C	0.16	0.280	0.068	3.79E-05	++++	1.32	0.89	0.80	1.00	GNA14	26785
rs690232	9	92422258	A	G	0.30	0.236	0.055	1.57E-05	+++-	1.27	0.93	0.99	1.00	DIRAS2	10325
rs10990103	9	104253849	T	C	0.28	0.220	0.056	8.07E-05	++++	1.25	0.96	0.90	0.99	CYLC2	543634
rs10760894	9	104259326	T	C	0.29	0.212	0.054	8.84E-05	++++	1.24	0.93	1.00	1.04	CYLC2	538157
rs2274899	9	113213385	A	T	0.19	-0.249	0.064	8.70E-05	----	0.78	0.93	0.78	1.01	KIAA0368	50592
rs10114818	9	120462359	A	G	0.04	0.666	0.167	6.71E-05	++++	1.95	0.68	0.51	0.82	DBC1	506369
rs10508257	10	3689729	A	G	0.07	0.382	0.098	9.05E-05	-+++	1.47	0.92	0.99	1.00	KLF6	121511
rs9664222	10	89328613	A	C	0.21	-0.267	0.062	1.61E-05	----	0.77	0.86	0.63	1.09	MINPP1	25489
rs2495702	10	102582965	T	C	0.35	-0.223	0.056	7.41E-05	----	0.80	0.87	0.68	0.99	PAX2	3277
rs3124736	10	115487102	A	G	0.03	0.835	0.192	1.41E-05	+++?	2.30	0.64	0.06	0.92	CASP7	6450
rs17606668	10	125361128	A	C	0.32	0.208	0.053	9.21E-05	++++	1.23	0.96	0.85	1.02	GPR26	54732
rs2583125	11	13051969	T	G	0.06	-0.413	0.104	7.05E-05	----	0.66	0.93	0.99	1.01	LOC644943	62746
rs2583124	11	13052483	T	C	0.07	-0.423	0.106	6.98E-05	----	0.66	0.95	0.82	1.01	LOC644943	63260
rs12364051	11	112810524	A	G	0.39	-0.206	0.051	5.66E-05	----	0.81	0.95	0.93	0.97	DRD2	24997
rs11607834	11	112966890	A	C	0.12	-0.322	0.080	5.08E-05	----	0.72	0.93	0.80	1.00	TMPRSS5	96592
rs11607852	11	112966927	G	C	0.12	-0.323	0.080	5.02E-05	----	0.72	0.93	0.80	1.00	TMPRSS5	96555
rs3923972	11	112967222	A	G	0.12	-0.323	0.080	4.98E-05	----	0.72	0.93	0.80	1.00	TMPRSS5	96260
rs17603387	11	112982797	C	T	0.12	-0.316	0.078	5.37E-05	----	0.73	0.96	0.88	1.00	TMPRSS5	80685
rs11601692	11	113006031	T	C	0.11	-0.308	0.078	8.18E-05	----	0.73	0.98	0.95	1.00	TMPRSS5	57451
rs11605737	11	113009346	C	T	0.11	-0.315	0.079	6.09E-05	----	0.73	0.98	0.95	1.00	TMPRSS5	54136
rs17531898	11	113012297	C	T	0.11	-0.321	0.079	5.44E-05	----	0.73	0.97	0.93	1.00	TMPRSS5	51185
rs11601402	11	113017732	T	A	0.11	-0.342	0.081	2.57E-05	----	0.71	0.95	0.86	1.00	TMPRSS5	45750
rs17610557	11	113018709	T	A	0.11	-0.346	0.082	2.32E-05	----	0.71	0.95	0.85	1.00	TMPRSS5	44773
rs17532157	11	113021980	T	G	0.12	-0.336	0.082	4.03E-05	----	0.71	0.89	0.75	0.99	TMPRSS5	41502
rs17532254	11	113024145	G	A	0.11	-0.346	0.082	2.23E-05	----	0.71	0.95	0.84	1.00	TMPRSS5	39337
rs11602504	11	113025332	G	A	0.11	-0.346	0.081	2.10E-05	----	0.71	0.95	0.86	1.00	TMPRSS5	38150
rs17532375	11	113026817	A	G	0.11	-0.345	0.081	2.12E-05	----	0.71	0.96	0.87	1.00	TMPRSS5	36665
rs17610915	11	113027800	C	T	0.11	-0.344	0.081	2.12E-05	----	0.71	0.96	0.88	1.00	TMPRSS5	35682
rs17532479	11	113028191	C	T	0.11	-0.343	0.081	2.15E-05	----	0.71	0.96	0.89	1.00	TMPRSS5	35291
rs11600717	11	113029072	A	G	0.11	-0.342	0.080	2.17E-05	----	0.71	0.97	0.90	1.00	TMPRSS5	34410
rs11602934	11	113029357	C	T	0.11	-0.338	0.080	2.30E-05	----	0.71	0.98	0.93	1.00	TMPRSS5	34125
rs11601890	11	113031358	T	C	0.12	-0.330	0.082	5.27E-05	----	0.72	0.88	0.76	0.94	TMPRSS5	32124

rs11607594	11	113031761	T	C	0.11	-0.332	0.079	2.56E-05	----	0.72	0.99	0.97	1.00	TMPRSS5	31721
rs11607622	11	113031875	T	C	0.11	-0.325	0.078	3.31E-05	----	0.72	0.93	0.99	1.00	TMPRSS5	31607
rs11607747	11	113032411	A	G	0.11	-0.333	0.079	2.39E-05	----	0.72	0.98	0.94	1.00	TMPRSS5	31071
rs11602134	11	113042120	T	G	0.11	-0.339	0.080	2.11E-05	----	0.71	0.96	0.86	1.00	TMPRSS5	21362
rs11606746	11	113042520	G	A	0.12	-0.336	0.079	2.39E-05	----	0.71	0.95	0.85	1.00	TMPRSS5	20962
rs1355202	11	113046231	T	C	0.11	-0.338	0.080	2.28E-05	----	0.71	0.95	0.85	1.00	TMPRSS5	17251
rs11605078	11	113047204	T	C	0.11	-0.342	0.081	2.19E-05	----	0.71	0.94	0.81	1.00	TMPRSS5	16278
rs11605096	11	113047320	A	C	0.12	-0.345	0.081	1.94E-05	----	0.71	0.93	0.77	1.00	TMPRSS5	16162
rs11600908	11	113047381	G	A	0.12	-0.344	0.081	1.96E-05	----	0.71	0.93	0.77	1.00	TMPRSS5	16101
rs11606211	11	113048743	A	C	0.12	-0.342	0.080	2.10E-05	----	0.71	0.94	0.80	1.00	TMPRSS5	14739
rs11606336	11	113049366	A	G	0.11	-0.345	0.081	2.04E-05	----	0.71	0.93	0.78	1.00	TMPRSS5	14116
rs11607704	11	113053259	C	T	0.12	-0.342	0.081	2.36E-05	----	0.71	0.93	0.76	1.00	TMPRSS5	10223
rs17611093	11	113053519	C	T	0.12	-0.342	0.081	2.37E-05	----	0.71	0.93	0.76	1.00	TMPRSS5	9963
rs17611127	11	113053884	C	T	0.12	-0.342	0.081	2.36E-05	----	0.71	0.93	0.75	1.00	TMPRSS5	9598
rs11604194	11	113058259	C	A	0.11	-0.345	0.081	2.20E-05	----	0.71	0.93	0.76	1.00	TMPRSS5	5223
rs17532884	11	113058827	T	C	0.11	-0.345	0.081	2.23E-05	----	0.71	0.93	0.77	1.00	TMPRSS5	4655
rs11607690	11	113066937	A	G	0.11	-0.319	0.081	7.41E-05	----	0.73	0.96	0.88	1.00	TMPRSS5	3455
rs11600570	11	113069222	A	G	0.11	-0.320	0.081	7.80E-05	----	0.73	0.96	0.88	1.00	TMPRSS5	5740
rs11601425	11	113075595	T	C	0.11	-0.320	0.081	8.48E-05	----	0.73	0.96	0.88	1.00	TMPRSS5	6710
rs10502177	11	113091121	G	C	0.11	-0.350	0.087	5.44E-05	----	0.70	0.88	0.58	1.00	TMPRSS5	8816
rs17612378	11	113095407	A	C	0.11	-0.351	0.087	5.15E-05	----	0.70	0.88	0.58	1.00	TMPRSS5	13102
rs11607718	11	113097218	A	G	0.11	-0.352	0.087	4.91E-05	----	0.70	0.87	0.58	1.00	ZW10	11902
rs11604887	11	113108019	C	T	0.11	-0.347	0.086	5.18E-05	----	0.71	0.89	0.62	1.00	ZW10	1101
rs17541000	11	113116948	G	A	0.11	-0.345	0.086	5.59E-05	----	0.71	0.89	0.63	1.00	ZW10	7828
rs17541153	11	113120832	C	G	0.11	-0.345	0.085	5.01E-05	----	0.71	0.89	0.62	1.00	ZW10	11712
rs11611975	12	17853947	A	G	0.17	0.261	0.065	5.41E-05	++++	1.30	0.97	0.92	1.00	FLJ22655	271122
rs10843049	12	28025611	T	C	0.18	-0.300	0.072	2.74E-05	----	0.74	0.75	0.23	1.01	PTHLH	9428
rs11049255	12	28027256	C	T	0.18	-0.299	0.072	2.80E-05	----	0.74	0.75	0.23	1.01	PTHLH	11073
rs11049256	12	28028740	A	G	0.18	-0.298	0.071	2.95E-05	----	0.74	0.76	0.23	1.01	PTHLH	12557
rs42294	12	28029136	G	T	0.23	-0.272	0.067	4.38E-05	----	0.76	0.73	0.21	0.98	PTHLH	12953
rs33083	12	28030579	A	G	0.19	-0.275	0.069	6.45E-05	----	0.76	0.71	0.26	1.00	PTHLH	14396
rs886347	13	52265459	A	G	0.48	-0.218	0.053	3.33E-05	----	0.80	0.88	0.67	0.98	PCDH8	50652
rs1333420	13	80654428	C	A	0.09	-0.382	0.090	2.18E-05	----	0.68	0.95	0.89	0.99	SPRY2	841341
rs7324138	13	80660001	C	T	0.09	-0.388	0.089	1.43E-05	----	0.68	0.92	0.95	1.00	SPRY2	846914
rs12854616	13	80660902	C	T	0.08	-0.388	0.093	3.28E-05	----	0.68	0.97	0.94	1.00	SPRY2	847815
rs12875277	13	80672711	T	A	0.08	-0.380	0.093	4.16E-05	----	0.68	0.98	0.95	1.00	SPRY2	859624

rs17073844	13	80673056	T	A	0.08	-0.379	0.093	4.19E-05	----	0.68	0.98	0.95	1.00	SPRY2	859969
rs7321904	13	80681190	T	C	0.07	-0.441	0.101	1.27E-05	----	0.64	0.90	0.86	0.96	SPRY2	868103
rs1333416	13	80691289	C	A	0.08	-0.379	0.095	6.67E-05	----	0.68	0.91	0.71	1.00	SPRY2	878202
rs17073955	13	80708859	T	C	0.08	-0.421	0.106	7.21E-05	----	0.66	0.75	0.20	0.99	SPRY2	895772
rs12891954	14	22424539	A	G	0.15	0.303	0.077	8.17E-05	++++	1.35	0.77	0.50	0.99	REM2	2190
rs7155742	14	22428444	G	A	0.28	0.237	0.055	1.65E-05	++++	1.27	1.00	1.00	1.00	REM2	1715
rs1543505	14	22432468	G	A	0.28	0.240	0.055	1.26E-05	++++	1.27	0.93	1.00	1.01	REM2	5739
rs4982702	14	22433172	G	A	0.28	0.238	0.055	1.51E-05	++++	1.27	1.00	1.00	1.00	REM2	6443
rs4982703	14	22433312	A	G	0.28	0.238	0.055	1.52E-05	++++	1.27	1.00	1.00	1.00	RBM23	6382
rs8012963	14	22435114	T	C	0.28	0.238	0.055	1.51E-05	++++	1.27	1.00	1.00	1.01	RBM23	4580
rs4982705	14	22436418	C	T	0.28	0.242	0.055	1.28E-05	++++	1.27	0.99	0.98	1.00	RBM23	3276
rs11625406	14	49585942	C	A	0.39	-0.234	0.054	1.71E-05	----	0.79	0.85	0.56	0.99	LOC196913	34177
rs11157721	14	49586464	T	C	0.39	-0.234	0.054	1.66E-05	----	0.79	0.85	0.56	1.00	LOC196913	33655
rs9323180	14	49588331	T	C	0.39	-0.227	0.054	2.95E-05	----	0.80	0.85	0.58	1.00	LOC196913	31788
rs1950705	14	49590156	C	T	0.39	-0.226	0.054	3.31E-05	----	0.80	0.85	0.56	0.99	LOC196913	29963
rs941604	14	49592824	A	G	0.38	-0.236	0.057	2.94E-05	----	0.79	0.80	0.44	0.98	LOC196913	27295
rs210332	14	53262222	C	T	0.19	0.284	0.067	2.28E-05	++++	1.33	0.79	0.50	1.00	BMP4	223982
rs234577	14	96169379	C	T	0.42	0.199	0.050	8.09E-05	++++	1.22	0.95	0.87	0.99	PAPOLA	66180
rs7170328	15	71190442	A	G	0.07	0.407	0.102	6.89E-05	-+++	1.50	0.93	0.80	0.99	NEO1	58515
rs16957640	15	71190747	T	C	0.07	0.411	0.102	5.85E-05	-+++	1.51	0.94	0.83	0.99	NEO1	58820
rs7177629	15	71199814	C	T	0.08	0.397	0.102	9.65E-05	-+++	1.49	0.86	0.74	0.93	NEO1	67887
rs6495056	15	71204618	G	A	0.07	0.416	0.102	4.87E-05	-+++	1.52	0.95	0.84	1.00	NEO1	72691
rs7163504	15	71208606	G	A	0.07	0.421	0.104	5.24E-05	-+++	1.52	0.93	0.79	1.00	NEO1	76679
rs2587748	15	98743231	G	A	0.40	-0.200	0.050	7.08E-05	----	0.82	0.99	0.96	1.00	LASS3	14892
rs2587736	15	98755969	C	T	0.40	-0.198	0.050	8.72E-05	----	0.82	0.98	0.94	1.02	LASS3	2154
rs12914235	15	98759896	T	C	0.40	-0.202	0.050	5.95E-05	----	0.82	0.99	0.96	1.02	LASS3	1773
rs1023782	15	98760366	G	T	0.40	-0.202	0.050	5.84E-05	----	0.82	0.99	0.96	1.02	LASS3	2243
rs7180354	15	98763870	T	C	0.40	-0.202	0.050	5.82E-05	----	0.82	0.99	0.98	1.02	LASS3	5747
rs1000290	15	98767132	C	T	0.40	-0.200	0.050	6.15E-05	----	0.82	0.93	1.00	1.02	LASS3	9009
rs7164184	15	98770934	A	G	0.40	-0.204	0.050	4.60E-05	----	0.82	1.00	0.99	1.02	LASS3	12811
rs1393943	15	98788724	A	T	0.42	-0.205	0.050	4.65E-05	----	0.81	0.97	0.91	1.02	LASS3	30601
rs2654602	15	98793158	T	C	0.40	-0.202	0.052	9.99E-05	----	0.82	0.94	0.84	0.98	LASS3	35035
rs2587803	15	98806337	C	T	0.40	-0.200	0.051	9.80E-05	----	0.82	0.94	0.82	1.00	LASS3	48214
rs1988459	15	98822837	C	T	0.42	-0.217	0.053	3.82E-05	----	0.81	0.88	0.63	1.02	LASS3	64714
rs8028050	15	98824226	A	G	0.44	-0.205	0.053	9.66E-05	----	0.81	0.89	0.63	1.01	LASS3	66103
rs8029244	15	98826098	A	G	0.49	-0.235	0.054	1.17E-05	----	0.79	0.86	0.54	1.02	LASS3	67975

rs8028803	15	98826193	C	T	0.49	-0.234	0.054	1.22E-05	----	0.79	0.86	0.54	1.02	LASS3	68070
rs12910887	15	98826470	T	C	0.49	-0.234	0.054	1.22E-05	----	0.79	0.86	0.54	1.02	LASS3	68347
rs12906592	15	98826772	C	T	0.50	-0.224	0.054	3.36E-05	----	0.80	0.84	0.51	1.02	LASS3	68649
rs1466931	15	98827874	A	T	0.50	-0.226	0.054	3.05E-05	----	0.80	0.84	0.51	1.02	LASS3	69751
rs1466930	15	98828233	C	G	0.50	-0.226	0.054	3.06E-05	----	0.80	0.84	0.51	1.02	LASS3	70110
rs11634135	15	98828554	G	A	0.50	-0.227	0.054	2.79E-05	----	0.80	0.84	0.51	1.03	LASS3	70431
rs1393942	15	98830020	C	T	0.50	-0.226	0.054	2.94E-05	----	0.80	0.84	0.52	1.02	LASS3	71897
rs12444419	16	69965976	G	T	0.32	-0.205	0.052	7.93E-05	+---	0.82	0.93	1.00	1.03	CALB2	15850
rs8046337	16	69966028	G	A	0.32	-0.205	0.052	7.55E-05	+---	0.81	1.00	1.00	1.03	CALB2	15815
rs11641122	16	69967621	T	G	0.32	-0.209	0.052	5.64E-05	+---	0.81	1.00	0.99	1.01	CALB2	14222
rs12935091	16	70082709	G	A	0.07	-0.477	0.112	2.00E-05	----	0.62	0.65	0.20	0.90	ZNF19	1954
rs4149497	16	70116471	A	G	0.12	-0.360	0.084	2.02E-05	----	0.70	0.77	0.32	0.99	CHST4	1090
rs766410	16	72645974	G	A	0.16	0.295	0.074	6.50E-05	++++	1.34	0.75	0.68	0.79	PSMD7	242207
rs2293776	16	76621852	G	C	0.30	-0.248	0.062	5.52E-05	----	0.78	0.76	0.33	0.93	CLEC3A	1647
rs4494555	16	76626102	G	C	0.34	-0.238	0.060	7.04E-05	----	0.79	0.76	0.25	0.97	CLEC3A	2603
rs1955389	16	76626465	T	C	0.33	-0.235	0.059	7.16E-05	----	0.79	0.78	0.25	1.00	CLEC3A	2966
rs1955390	16	76626535	C	T	0.33	-0.235	0.059	7.29E-05	----	0.79	0.78	0.25	1.00	CLEC3A	3036
rs2344495	16	76627501	A	G	0.33	-0.234	0.059	7.67E-05	----	0.79	0.78	0.26	1.00	CLEC3A	4002
rs11642341	16	76631895	G	T	0.33	-0.235	0.059	7.21E-05	----	0.79	0.78	0.27	1.00	CLEC3A	8396
rs11642677	16	76632910	G	A	0.33	-0.234	0.059	7.54E-05	----	0.79	0.79	0.28	1.00	CLEC3A	9411
rs2344922	16	76642909	G	C	0.33	-0.221	0.056	8.12E-05	----	0.80	0.85	0.50	1.00	CLEC3A	19410
rs8045479	16	76643862	G	C	0.32	-0.228	0.057	6.55E-05	----	0.80	0.83	0.47	0.98	CLEC3A	20363
rs8044195	16	76643897	A	G	0.33	-0.222	0.056	8.53E-05	----	0.80	0.85	0.49	1.00	CLEC3A	20398
rs4369682	16	76645062	G	C	0.33	-0.221	0.056	8.62E-05	----	0.80	0.85	0.49	1.00	CLEC3A	21563
rs11150040	16	76648483	G	C	0.33	-0.217	0.056	9.82E-05	----	0.81	0.86	0.54	1.00	CLEC3A	24984
rs12952455	17	28582692	A	G	0.26	-0.249	0.058	1.87E-05	----	0.78	0.90	0.79	0.99	ACCN1	61427
rs9972933	17	28589381	T	C	0.23	-0.255	0.058	1.10E-05	----	0.77	0.99	0.98	1.03	ACCN1	54738
rs9972931	17	28589889	A	G	0.23	-0.253	0.058	1.25E-05	----	0.78	0.93	1.00	1.03	ACCN1	54230
rs16972414	18	35709920	G	A	0.30	-0.234	0.055	1.96E-05	----	0.79	0.94	0.85	0.99	PIK3C3	2079276
rs12967168	18	35728048	T	A	0.29	-0.226	0.054	3.01E-05	----	0.80	0.98	0.93	1.00	PIK3C3	2061148
rs11082159	18	35732656	T	C	0.29	-0.227	0.054	2.60E-05	----	0.80	0.92	0.96	1.00	PIK3C3	2056540
rs12956332	18	63440863	A	G	0.17	-0.354	0.088	5.51E-05	----	0.70	0.53	0.42	0.61	DSEL	105916
rs12958645	18	63453481	A	C	0.17	-0.283	0.073	9.86E-05	----	0.75	0.82	0.62	0.93	DSEL	118534
rs12963356	18	63454257	G	A	0.19	-0.255	0.065	9.15E-05	----	0.78	0.84	0.61	1.00	DSEL	119310
rs7237695	18	63454601	T	C	0.18	-0.275	0.070	7.49E-05	----	0.76	0.87	0.67	0.97	DSEL	119654
rs12605030	18	63457377	T	C	0.20	-0.278	0.069	5.73E-05	----	0.76	0.78	0.60	0.89	DSEL	122430

rs12965352	18	63461303	A	G	0.16	-0.304	0.075	4.80E-05	----	0.74	0.80	0.66	0.91	DSEL	126356
rs2440514	18	63469463	G	C	0.19	-0.261	0.067	8.46E-05	----	0.77	0.88	0.77	0.97	DSEL	134516
rs2423969	20	15599436	G	A	0.43	-0.201	0.050	6.57E-05	----	0.82	0.88	0.76	1.00	MACROD2	382403
rs2423970	20	15599466	A	G	0.43	-0.201	0.051	7.24E-05	----	0.82	0.94	0.76	0.99	MACROD2	382373
rs1362512	20	15599887	A	G	0.43	-0.201	0.051	7.25E-05	----	0.82	0.94	0.76	0.99	MACROD2	381952
rs6035189	20	18935154	T	C	0.20	-0.282	0.070	6.22E-05	?---	0.75	0.73	0.00	0.99	C20orf79	192121
rs1535487	20	18937951	A	C	0.20	-0.297	0.073	4.69E-05	?---	0.74	0.71	0.00	0.95	C20orf79	194918
rs6004864	22	24709837	C	T	0.04	0.675	0.170	6.94E-05	+++?	1.96	0.56	0.05	0.84	MYO18B	47170
rs6004867	22	24710058	T	C	0.04	0.650	0.161	5.43E-05	+++?	1.92	0.63	0.08	0.89	MYO18B	46949
rs16981163	22	24719510	A	G	0.04	0.640	0.158	5.02E-05	+++?	1.90	0.73	0.17	0.99	MYO18B	37497
rs8136667	22	24726280	T	C	0.04	0.629	0.159	7.50E-05	+++-	1.88	0.73	0.18	0.99	MYO18B	30727
rs9620582	22	24727504	C	G	0.04	0.676	0.165	4.11E-05	+++?	1.97	0.73	0.17	0.99	MYO18B	29503
rs6004887	22	24736136	T	G	0.04	0.652	0.167	9.31E-05	+++?	1.92	0.73	0.17	1.00	MYO18B	20871
rs6004890	22	24736991	G	A	0.04	0.653	0.167	9.23E-05	+++?	1.92	0.73	0.17	1.00	MYO18B	20016

Supplemental Table 3. Study-specific results with the initial 4-study CHARGE meta-analysis and 6-study CHARGE+Danish and Leiden study meta analyses, ordered by p-value.

Hit Number	Marker Name	Chr	position	Allele minor	Allele major	Study	r^2	beta	se	p-value	OR
1	rs4443878	1	238971041	T	C	AGES- Reykjavik	0.79	-0.77	0.75	3.01E-01	0.46
						Rotterdam	0.92	-0.96	0.25	1.43E-04	0.38
						FHS	0.44	-0.72	0.36	4.49E-02	0.49
						CHS	0.31	-1.04	0.51	3.98E-02	0.35
						Four study meta-analysis		-0.89	0.18	1.33E-06	0.41
						Danish	g	0.06	0.15	7.02E-01	1.06
						Leiden	g	0.25	0.21	2.38E-01	1.28
						Six study meta-analysis		-0.19	0.10	6.78E-02	0.83
2	rs9825185	3	196415923	C	A	AGES- Reykjavik	g	0.20	0.25	4.21E-01	1.22
						Rotterdam	0.99	0.24	0.11	2.48E-02	1.28
						FHS	0.80	0.58	0.23	1.09E-02	1.78
						CHS	g	0.55	0.15	1.72E-04	1.73
						Four study meta-analysis		0.36	0.08	2.48E-06	1.44
						Danish	g	-0.07	0.07	3.01E-01	0.93
						Leiden	g	-0.03	0.10	7.57E-01	0.97
						Six study meta-analysis		0.09	0.05	4.45E-02	1.10
3	rs954551	6	102886028	G	A	AGES- Reykjavik	g	-0.58	0.20	4.07E-03	0.56
						Rotterdam	1.00	-0.28	0.09	1.16E-03	0.76
						FHS	0.93	-0.07	0.15	6.67E-01	0.94
						CHS	g	-0.24	0.10	1.52E-02	0.79
						Four study meta-analysis		-0.26	0.06	5.33E-06	0.77
4	rs7624691	3	138345769	C	T	AGES- Reykjavik	g	-0.32	0.18	7.99E-02	0.73
						Rotterdam	1.00	-0.21	0.08	5.78E-03	0.81
						FHS	0.97	-0.26	0.13	4.52E-02	0.77
						CHS	g	-0.20	0.09	1.92E-02	0.82
						Four study meta-analysis		-0.22	0.05	8.83E-06	0.80
						Danish	g	0.02	0.05	6.36E-01	1.02
						Leiden	g	0.13	0.07	6.30E-02	1.14
						Six study meta-analysis		-0.05	0.03	9.18E-02	0.95
5	rs10888267	1	246126046	C	T	AGES- Reykjavik	g	0.64	0.18	4.62E-04	1.90
						Rotterdam	g	0.16	0.08	3.94E-02	1.17
						FHS	g	0.30	0.12	1.01E-02	1.34
						CHS	g	0.16	0.09	6.78E-02	1.17
						Four study meta-analysis		0.22	0.05	9.66E-06	1.25
6	rs9972933	17	28589381	T	C	AGES- Reykjavik	1.00	-0.18	0.22	4.28E-01	0.84
						Rotterdam	1.00	-0.20	0.09	2.75E-02	0.82
						FHS	g	-0.44	0.13	4.85E-04	0.64
						CHS	0.98	-0.22	0.10	3.45E-02	0.80
						Four study meta-analysis		-0.26	0.06	1.10E-05	0.77
						Danish	g	-0.05	0.06	4.04E-01	0.95
						Leiden	g	0.07	0.08	3.74E-01	1.08
						Six study meta-analysis		-0.11	0.04	3.08E-03	0.89
7	rs2739532	4	190944424	C	G	AGES- Reykjavik	<0.10				1.00
						Rotterdam	0.51	0.41	0.12	6.05E-04	1.51
						FHS	<0.10				1.00
						CHS	0.50	0.37	0.14	5.96E-03	1.45
						Four study meta-analysis		0.40	0.09	1.14E-05	1.48
8	rs8029244	15	98826098	A	G	AGES- Reykjavik	0.91	-0.51	0.18	4.94E-03	0.60
						Rotterdam	1.00	-0.21	0.08	6.04E-03	0.81
						FHS	g	-0.31	0.11	7.61E-03	0.74
						CHS	0.54	-0.10	0.12	3.89E-01	0.90
						Four study meta-analysis		-0.23	0.05	1.17E-05	0.79
						Danish	g	-0.06	0.05	1.96E-01	0.94
						Leiden	g	0.06	0.07	3.70E-01	1.07
						Six study meta-analysis		-0.10	0.03	2.03E-03	0.90

9	rs16850255	1	175085164	C	T	AGES- Reykjavik	0.99	-0.21	0.21	3.11E-01	0.81
						Rotterdam	1.00	-0.13	0.09	1.52E-01	0.87
						FHS	0.98	-0.45	0.14	1.28E-03	0.64
						CHS	0.44	-0.56	0.16	3.09E-04	0.57
						Four study meta-analysis		-0.29	0.07	1.18E-05	0.75
						Danish	g	0.04	0.06	5.66E-01	1.04
						Leiden	g	0.04	0.08	6.43E-01	1.04
						Six study meta-analysis		-0.08	0.04	4.13E-02	0.92
10	rs1543505	14	22432468	G	A	AGES- Reykjavik	g	0.41	0.20	4.41E-02	1.50
						Rotterdam	1.00	0.16	0.09	6.21E-02	1.17
						FHS	1.00	0.31	0.13	1.73E-02	1.36
						CHS	g	0.26	0.09	5.38E-03	1.30
						Four study meta-analysis		0.24	0.06	1.26E-05	1.27
						Danish	g	0.05	0.06	3.95E-01	1.05
						Leiden	g	0.00	0.08	9.53E-01	1.00
						Six study meta-analysis		0.12	0.04	9.18E-04	1.12
11	rs7321904	13	80681190	T	C	AGES- Reykjavik	0.96	-0.65	0.32	3.79E-02	0.52
						Rotterdam	0.93	-0.39	0.16	1.48E-02	0.68
						FHS	0.90	-0.67	0.25	6.96E-03	0.51
						CHS	0.86	-0.32	0.18	6.61E-02	0.72
						Four study meta-analysis		-0.44	0.10	1.27E-05	0.64
						Danish	g	0.11	0.09	2.54E-01	1.11
						Leiden	g	0.16	0.13	2.37E-01	1.17
						Six study meta-analysis		-0.08	0.06	1.79E-01	0.92
12	rs17401847	1	20111053	G	A	AGES- Reykjavik	0.99	0.21	0.25	3.98E-01	1.24
						Rotterdam	0.96	0.31	0.11	5.31E-03	1.37
						FHS	0.85	0.28	0.17	1.05E-01	1.32
						CHS	0.80	0.38	0.13	3.95E-03	1.47
						Four study meta-analysis		0.32	0.07	1.35E-05	1.38
						Danish	g	0.01	0.07	8.84E-01	1.01
						Leiden	g	-0.08	0.10	4.28E-01	0.92
						Six study meta-analysis		0.11	0.05	1.47E-02	1.12
13	rs3124736	10	115487102	A	G	AGES- Reykjavik	0.87	1.24	0.67	6.27E-02	3.47
						Rotterdam	0.86	0.73	0.25	3.09E-03	2.08
						FHS	g	0.93	0.34	7.05E-03	2.53
						CHS	<0.1				1.00
						Meta-analysis		0.83	0.19	1.41E-05	2.30
14	rs690232	9	92422258	A	G	AGES- Reykjavik	g	0.40	0.19	2.96E-02	1.50
						Rotterdam	1.00	0.39	0.08	2.08E-06	1.48
						FHS	0.99	0.23	0.14	1.13E-01	1.26
						CHS	g	-0.01	0.09	9.35E-01	0.99
						Four study meta-analysis		0.24	0.05	1.57E-05	1.27
15	rs9664222	10	89328613	A	C	AGES- Reykjavik	g	-0.12	0.21	5.62E-01	0.88
						Rotterdam	0.99	-0.16	0.09	8.89E-02	0.85
						FHS	0.63	-0.17	0.16	3.06E-01	0.85
						CHS	g	-0.47	0.11	6.72E-06	0.62
						Four study meta-analysis		-0.27	0.06	1.61E-05	0.77
						Danish	g	-0.08	0.06	1.87E-01	0.92
						Leiden	g	-0.28	0.09	1.44E-03	0.76
						Six study meta-analysis		-0.19	0.04	6.77E-07	0.82
16	rs11157721	14	49586464	T	C	AGES- Reykjavik	0.75	-0.19	0.20	3.39E-01	0.83
						Rotterdam	g	-0.23	0.08	3.14E-03	0.79
						FHS	g	-0.36	0.11	1.51E-03	0.70
						CHS	0.56	-0.12	0.12	3.00E-01	0.89
						Four study meta-analysis		-0.23	0.05	1.66E-05	0.79
						Danish	g	-0.05	0.05	3.79E-01	0.95
						Leiden	g	0.03	0.07	6.48E-01	1.03
						Six study meta-analysis		-0.10	0.03	2.50E-03	0.90
17	rs4690810	4	166500130	C	T	AGES- Reykjavik	0.94	-0.31	0.18	8.91E-02	0.74

						Rotterdam	0.97	-0.12	0.08	1.24E-01	0.88
						FHS	0.97	-0.40	0.14	3.79E-03	0.67
						Four study meta-analy	0.82	-0.29	0.10	3.61E-03	0.75
						Meta-analysis		-0.23	0.05	1.86E-05	0.79
						Danish	g	0.02	0.05	7.09E-01	1.02
						Leiden	g	0.07	0.07	3.81E-01	1.07
						Six study meta-analysis		-0.07	0.03	4.42E-02	0.93
18	rs11605096	11	113047320	A	C	AGES- Reykjavik	1.00	-0.53	0.25	3.57E-02	0.59
						Rotterdam	1.00	-0.34	0.12	4.74E-03	0.71
						FHS	0.99	-0.38	0.18	3.56E-02	0.68
						CHS	0.77	-0.25	0.16	1.21E-01	0.78
						Four study meta-analysis		-0.34	0.08	1.94E-05	0.71
19	rs16972414	18	35709920	G	A	AGES- Reykjavik	0.94	-0.29	0.21	1.60E-01	0.75
						Rotterdam	0.99	-0.24	0.08	4.45E-03	0.79
						FHS	0.95	-0.22	0.12	7.25E-02	0.80
						CHS	0.85	-0.23	0.10	2.49E-02	0.80
						Four study meta-analysis		-0.23	0.05	1.96E-05	0.79
20	rs12935091	16	70082709	G	A	AGES- Reykjavik	0.81	-0.20	0.30	5.10E-01	0.82
						Rotterdam	0.81	-0.52	0.16	1.04E-03	0.59
						FHS	0.90	-0.44	0.22	4.25E-02	0.64
						CHS	0.20	-0.75	0.36	3.49E-02	0.47
						Four study meta-analysis		-0.48	0.11	2.00E-05	0.62
						Danish	g	0.01	0.11	9.46E-01	1.01
						Leiden	g	-0.15	0.18	3.86E-01	0.86
						Six study meta-analysis		-0.22	0.07	2.09E-03	0.80
21	rs210332	14	53262222	C	T	AGES- Reykjavik	g	0.33	0.24	1.59E-01	1.39
						Rotterdam	0.90	0.35	0.10	4.51E-04	1.42
						FHS	0.50	0.06	0.23	7.83E-01	1.07
						CHS	g	0.24	0.11	2.43E-02	1.28
						Four study meta-analysis		0.28	0.07	2.28E-05	1.33
22	rs17369174	8	76128602	G	T	AGES- Reykjavik	1.00	-0.44	0.28	1.11E-01	0.64
						Rotterdam	1.00	-0.35	0.12	4.53E-03	0.71
						FHS	1.01	-0.57	0.20	5.13E-03	0.57
						CHS	0.69	-0.21	0.18	2.56E-01	0.81
						Four study meta-analysis		-0.37	0.09	2.30E-05	0.69
						Danish	g	-0.02	0.10	8.31E-01	0.98
						Leiden	g	0.17	0.13	2.10E-01	1.18
						Six study meta-analysis		-0.15	0.06	1.38E-02	0.86
23	rs6721003	2	166931758	A	G	AGES- Reykjavik	1.00	0.23	0.18	1.91E-01	1.26
						Rotterdam	1.00	0.11	0.08	1.35E-01	1.12
						FHS	1.01	0.24	0.12	3.58E-02	1.27
						CHS	0.98	0.31	0.09	4.12E-04	1.36
						Four study meta-analysis		0.21	0.05	2.44E-05	1.23
						Danish	g	0.01	0.05	8.94E-01	1.01
						Leiden	g	0.00	0.07	9.46E-01	1.00
						Six study meta-analysis		0.09	0.03	5.89E-03	1.09
24	rs4734457	8	101573533	A	C	AGES- Reykjavik	0.61	0.30	0.30	3.15E-01	1.35
						Rotterdam	0.54	0.50	0.17	3.20E-03	1.66
						FHS	0.21	0.98	0.46	3.38E-02	2.67
						CHS	0.11	1.06	0.41	9.09E-03	2.88
						Four study meta-analysis		0.56	0.13	2.47E-05	1.75
						Danish	g	-0.06	0.08	4.82E-01	0.95
						Leiden	g	0.07	0.11	5.36E-01	1.07
						Six study meta-analysis		0.10	0.06	9.80E-02	1.10

Notes:

beta (log OR) and OR are effect for each additional minor allele

r^2 is an estimate of imputation quality. It is the estimated alleleic correlation between the imputed genotypes and the true genotypes. Genotyped SNPs are indicated with a "g".

Abbreviations: chromosome, Chr; standard error, se; odds ratio, OR; Age, Gene/Environment Susceptibility-Reykjavik Study, AGES- Reykjavik; Cardiovascular Health Study, CHS; Framingham Heart Study, FHS; Rotterdam Study, Rotterdam; Leiden Longevity Study, Leiden; Danish 1905 cohort, Danish; adenine, A; cytocine, C; guanine, G; thymine, T;

CHAPTER 2.2

A Genome-Wide Association Study of Aging

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Neurobiology of Aging. 2011 Nov;32(11):2109.e15-28

Abstract

Human longevity and healthy aging show moderate heritability (20-50%). We conducted a meta-analysis of genome-wide association studies from nine studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium for two outcomes: a) all-cause mortality and b) survival free of major disease or death. No single nucleotide polymorphism (SNP) was a genome-wide significant predictor of either outcome ($p < 5 \times 10^{-8}$). We found fourteen independent SNPs that predicted risk of death, and eight SNPs that predicted event-free survival ($p < 10^{-5}$). These SNPs are in or near genes that are highly expressed in the brain (*HECW2*, *HIP1*, *BIN2*, *GRIA1*), genes involved in neural development and function (*KCNQ4*, *LMO4*, *GRIA1*, *NETO1*) and autophagy (*ATG4C*), and genes that are associated with risk of various diseases including cancer and Alzheimer's disease. In addition to considerable overlap between the traits, pathway and network analysis corroborated these findings. These findings indicate that variation in genes involved in neurological processes may be an important factor in regulating aging free of major disease and achieving longevity.

Introduction

The recent, remarkable extension of life expectancy is largely attributed to the postponement of mortality at old age (1-2). The years of life gained in the older population residing in developed nations are a success story of public health measures and improved health care. In addition to such external factors, longevity and healthy aging consistently show a modest heritability between 20 to 50% and aging associated genetic research may provide further insights into the mechanisms of aging (3-5). It has been postulated that genes involved in pathways associated with aging identified in animal models, such as IGF-insulin signalling, regulation of lipoprotein metabolism, the mTOR pathway, and the oxidative stress response may also influence survival to old or even exceptionally old age in humans (6-8). However, in humans, common variants within genes involved in these pathways have not been consistently associated with lifespan (6-7, 9-10).

The lack of success in the identification of genes related to aging in humans may be due to the complexity of the phenotype. One approach to investigate aging and longevity is to compare frequencies of genetic variants between nonagenarians or centenarians and the general population. This approach led to the discovery of an association between *APOE* (11-13) and more recently *FOXO3A* (14-18) and human aging and longevity. However, a recent GWAS of individuals reaching the age of 90 or older failed to identify genome-wide significant variants (19).

Prospective follow-up studies with a continuous outcome such as time to death are more powerful than case-control analyses. A study of time to death simultaneously addresses the effects of genetic variation related to life span, the progression towards death, and disease specific mortality. This design has been successfully applied in animal models (6, 20) and also in human genetics research of blood pressure (21-23), a trait with heritability similar to longevity, where examination of a continuous outcome has been more successful in identifying genetic loci than studies that have solely used hypertension as a dichotomous trait. Frailty and survival free of disease have been suggested as more promising phenotypes for studies of aging since mortality is a very heterogeneous outcome caused by multiple chronic conditions (9).

This study addresses the genetics of aging in a broad, sequential way using data from cohort studies participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. First, we aimed to identify SNPs associated with all cause mortality (time to death) in a hypothesis-free GWAS in ~ 25,000 unselected persons of European ancestry. Second, we performed GWAS of time to event, defined by major incident

events (myocardial infarction, heart failure, stroke, dementia, hip fracture or cancer) or death, as an alternative phenotype for healthy aging. Last, we analyzed the SNPs along with their respective most likely associated genes identified in the GWAS meta-analyses to identify pathways and networks associated with aging and longevity.

Methods

Participants

The participants are of recent European ancestry and stem from cohorts of the CHARGE Consortium (24). All cohorts are follow-up studies periodically assessing the health and vital status of their participants. Although some of the cohorts included multiple ethnic groups, only data from self-reported Caucasians was used. In addition, population structure was assessed using principal components in each CHARGE study and outliers were removed. Any remaining within-study structure was adjusted for using appropriate methods.(25) All participants included in this analysis were at least 55 years of age at the time of blood draw for DNA and provided written informed consent. A brief description of each population is given in the Supplementary Information.

Phenotype

We conducted a survival analysis, adjusted for age at baseline and sex, to model continuous time to death or end of follow-up in 25,007 participants (deceased ("cases") = 8,444, mean follow-up time = 10.6 (SD 5.4) years) that were older than 55 years at baseline. As research demonstrated that the likelihood of incident disease is genetically determined, we defined a second phenotype: survival free of major disease or mortality (9, 26-27). The outcome was defined as time to the first of the following adjudicated events: myocardial infarction, heart failure, stroke, dementia, hip fracture, cancer, or death. For this analysis, participants at baseline were older than 55 years of age and free of any of the aforementioned conditions. Inclusion in the study required complete follow-up information on mortality and at least 4 out of 6 of the health conditions. Genome-wide information on polymorphisms was available for 16,995 participants free of disease at the beginning of the study. These participants were followed for 8.8 (SD 5.7) years and we registered 7,314 major events.

Genotyping and Imputation

As different genotyping platforms were used across studies, we imputed to 2.5 million SNPs using the HapMap 22 CEU (build 36) genotyped samples as a reference. For details on the study specific quality control procedures for genotyping and imputation please consult Table S1 in the Supplementary Information (SI).

Statistical Analysis

We used the semi-parametric Cox proportional hazard to model time to event for both phenotypes in each study. Follow-up time since baseline was used as time scale. An additive genetic model was used in this analysis. We subsequently combined the individual study results in a meta-analysis using a fixed effects model that combined the study specific

regression parameters and standard errors using inverse variance weighting. We included SNPs that had a minor allele frequency (MAF) of at least 1% and an imputation quality ratio (28) (equivalent to the MaCH r^2 statistic (29)) of at least 0.3. The study specific inflation factors (λ_{GC}) were computed using the set of chi-square statistics used for the meta-analysis for each study. The inflation factor is computed as the median of all chi-square statistics divided by the expected median of the statistics (approximately 0.456) for a chi-square distribution with 1 degree of freedom. SNP associations at $p<5 \times 10^{-8}$ were considered to be genome-wide significant. SNPs with $p<5 \times 10^{-5}$ were considered suggestive associations. The combined meta-analysis hazard ratio (HR) can be interpreted as the increase in the risk of dying or having a major event during follow-up per additional copy of the coded allele. Power analysis revealed 80% statistical power to detect SNPs with a minor allele frequency of 5% and relative risk of 1.10 using a nominal significance level of 0.05 (Supplementary Table 2).

In addition, we incorporated gene annotation information, a technique that has successfully been applied in the field of aging research (30-31). Protein ANalysis THrough Evolutionary Relationships (PANTHER)(32-33) and Ingenuity Pathway Analysis (IPA) (www.ingenuity.com) were used for identification and classification of networks, pathways, biological processes and molecular functions of the genes identified in this study. For both phenotypes we generated lists of candidate genes. These genes were the closest reference genes to the SNPs associated with the outcome at $p < 1 \times 10^{-3}$. PANTHER compares these gene lists to the reference list using the binomial test for each molecular function, biological process, or pathway term. IPA builds networks by searching the Ingenuity Pathways Knowledge Base for interactions between the identified genes and all other gene objects stored in the knowledge base.

Results

We conducted a meta-analysis of GWAS on time to death adjusted for baseline age and sex in participants of European origin, 55 years of age or older from nine longitudinal cohort studies participating in the CHARGE Consortium (24). In total, we observed 8,444 deaths (mean age at death: 81.1, Standard Deviation (SD) 8.4)) in 25,007 participants (55% female) after an average follow up of 10.6 (SD 5.4) years. Descriptive characteristics of participants and Manhattan plots showing genome wide p-values for association are displayed in the Supplementary Information, (Figure S1, Tables S3-4). The quantile-quantile plot (Q-Q plot) of observed versus expected p-values showed only a small deviation from the null hypothesis, indicating no significant population stratification (Figure 1a, $\lambda_{GC} = 1.066$). Although there were no genome-wide significant findings ($p < 5 \times 10^{-8}$), 14 independent SNPs were associated with time to death at a suggestive threshold of $p < 1 \times 10^{-5}$ (Table 1). Among these SNPs, rs4936894 (chromosome 11, near the von Willebrand factor A domain containing 5A gene (*VWA5A*)) had the strongest association with time to death ($p = 3.4 \times 10^{-7}$). We sought replication for 5 of the 14 top SNPs with the strongest association with time to death in 4 independent samples (n=10,411, deaths= 1,295) of the same ancestry. None of the SNPs were consistently associated with time to death at a nominally significant level of $p < 0.05$ across all replication samples (Table S5-S8). In the combined meta-analysis of the discovery and replication studies only rs1425609 in the vicinity of otolin-1 (*OTOL1)* showed a stronger association (1.61×10^{-6}).

Likewise, no genome-wide significant findings were identified in the time to event analysis following 16,995 participants free of disease at baseline and registering 7,314 events over an average of 8.8 (SD 5.7) years of follow-up (Table 2). Events included incident myocardial infarction, heart failure, stroke, dementia, hip fracture, and cancer or death. The Q-Q plot (Figure 1b, $\lambda_{GC} = 1.019$) showed no evidence of inflation of type I error. In total, there were 8 independent SNPs associated with event-free survival at $p < 10^{-5}$. The SNP with the strongest association was rs10412199 (chromosome 19, $p = 3.02 \times 10^{-6}$), which is in close proximity to ataxia, cerebellar, Cayman type (*ATCAY*). Additional descriptive information including definitions of each event and association results with $p < 10^{-4}$ are provided in the Figure S2, Tables S9-S12.

As both phenotypes may provide different but complimentary information about the aging process, we evaluated the overlap between their association results (Table 3). Interpretation of the overlap between the phenotypes requires caution as both phenotypes are correlated, nevertheless it helps to focus on specific loci and put them into the context of

aging. From the 14 loci passing the pre-specified, suggestive threshold of $p < 1 \times 10^{-5}$ in the time to death analysis, 5 had corresponding SNPs within 500kb distance, in linkage disequilibrium (LD, $r^2 > 0.1$) associated with $p < 1 \times 10^{-4}$ and the same overall direction of the effect in the time to event analysis. These 5 regions were in the vicinity of the following genes: *OTOL1* (3q26.1), bridging integrator 2 (*BIN2*, 12q13), ATG4 autophagy related 4 homolog C (*ATG4C*, 1p31.3), origin recognition complex, subunit 5-like (*ORC5L*, 7q22.1), and potassium voltage-gated channel, KQT-like subfamily, member 4 (*KCNQ4*, 1p34). Similarly, in the time to event analysis three of the eight top SNPs showed considerable overlap and the same direction of effect in the time to death analysis. These SNPs were close to the following genes: MDS1 and EVI1 complex locus (*MECOM*, 3q24-q28), succinate-CoA ligase, ADP-forming, beta subunit (*SUCLA2*, 13q12.2-q13.3), and ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (*ST3GAL3*, 1p34.1).

Finally, we evaluated candidate genes for aging by identification and classification of networks, pathways, biological processes and molecular functions. The candidate genes were derived from the meta-analyses of GWAS and included the reference genes closest to the SNPs associated with $p < 1 \times 10^{-3}$ (time to death: 862 genes, time to event: 704 genes). We used PANTHER (32-34) and Ingenuity Pathway Analysis (IPA) software (www.ingenuity.com) for these analyses. PANTHER compares these gene lists to the reference list using the binomial test for each molecular function, biological process, or pathway term. IPA builds networks by searching the Ingenuity Pathways Knowledge Base for interactions between the identified genes and all other gene objects stored in the knowledge base.

For the analysis of time to death, the relevant biological processes overrepresented in the PANTHER analysis were *developmental processes, neuronal activities, signal transduction, neurogenesis, ectoderm development, and cell adhesion*. For the analysis of time to incident event, *developmental processes and neuronal activities* were overrepresented among other biological process (Table 4). The analyses also highlighted the Wnt signalling pathway. The Wnt signalling pathway is ubiquitous and know to be involved in cancer but also plays an important role in the early stages of the development of the central nervous system, in synaptic formation by axon guidance, and in modulating fibrosis during muscle repair scored high in both traits under study (35-38). For extended tables see Supplementary Information Table S13 and Table S14. In addition, Ingenuity identified one network with $p = 10^{-31}$ containing 26 genes involved in processes related to nervous system development and function for the analysis of time to death (Figure 2) and one network with $p = 10^{-40}$ containing

28 genes involved in cellular function and development for time to event (Supplementary Information, Figure S3).

IPA analysis highlighted the following genes associated with the time to death trait: *NTRK2* (neurotrophic tyrosine kinase, receptor, type 2), - a member of the neurotrophic tyrosine receptor kinase family. This kinase is a membrane-bound receptor that, upon neurotrophin binding, phosphorylates itself and members of the MAPK pathway. Signaling through this kinase leads to cell differentiation. Second in line were *NCAM1* (neural cell adhesion molecule 1), - a cytoskeletal binding protein, *GRID2* (glutamate receptor, ionotropic, delta 2), - a relatively new member of the family of ionotropic glutamate receptors which are the predominant excitatory neurotransmitter receptors in the mammalian brain, and have a role in neuronal apoptotic death, and *RIMS1* (regulating synaptic membrane exocytosis 1), which regulates synaptic vesicle exocytosis and may be part of the protein scaffold of the cell.

Among the genes that were highlighted through the IPA analysis in the analysis of time to event was *MYC* (v-myc myelocytomatosis viral oncogene homolog), - a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. *MYC* functions as a transcription factor that regulates transcription of specific target genes. Second in line were *E2F1* (E2F transcription factor 1), *EGFR* (epidermal growth factor receptor), and *CEBPA* (CCAAT/enhancer binding protein (C/EBP), alpha). *EF21*, a transcription factor, plays a crucial role in the control of cell cycle and action of tumor suppressor proteins, can mediate both cell proliferation and p53-dependent/independent apoptosis. *EGFR* is a transmembrane glycoprotein that serves as a receptor for members of the epidermal growth factor family and supports cell proliferation. *CEBP-Alpha*, a bZIP transcription factor, can bind as a homodimer to certain promoters and enhancers. *CEBPA* also forms heterodimers with the related proteins CEBP-beta and CEBP-gamma and modulates the expression of leptin, interacts with *CDK2* and *CDK4*, and thereby inhibits these kinases and causes growth arrest in cultured cells.

Discussion

In our analyses of over 25,000 individuals of 55 years and older followed for an average of 11 years, we did not identify genome-wide significant associations for all-cause mortality and survival free of major diseases. However, both traits highlighted loci with suggestive significance that were in the neighbourhood of genes related to neural regulation. In addition, our pathway and network analyses identified an enrichment of genes associated with cellular and neural development and function, and cell communication that may contribute to variation in human aging. Brain development might be responsible for the creation of redundancy in brain circuitry, which is associated with functional reserve and resiliency. Brain function regulates most of the compensatory strategy supporting maintenance of homeostatic equilibrium. Both of these processes are essential to healthy aging and longevity.

Several explanations are possible for the lack of genome-wide significant findings. First, mortality is arguably one of the most complex phenotypes, and several trajectories towards extreme old age have been identified (39). Multiple genes could mediate the aging process but would have their effects through numerous different pathophysiological processes and diseases that act as intermediate factors on the pathway to death (31). Therefore, any common variation in genes associated with aging probably has a small effect.

Second, the largely negative findings of this and other studies contrast with the intriguing animal studies of longevity. Very large effects of single genes on lifespan have indeed been observed in laboratory animals, but humans often have several homologues of these genes which might significantly differ in function or compensate for mutated genes through redundant mechanisms (10). This could explain why our top findings did not include genes in these pathways found in animal models. Animal models also represent genetically homogenous populations and are exposed to controlled environmental influences. The lack of replication of animal model findings in humans suggests that the use of knock out animals may not provide the optimal approach to understanding the variation in survival in humans as interactions with environmental factors may obscure the associations and prevent the identification of loci in humans.

Third, our study is based on common genetic variants and therefore we cannot exclude effects due to low frequency and rare variants (< 5%) or due to the presence of structural variation, such as copy number polymorphisms. Our discovery set may lack the power to identify all the relevant loci, even though we had sufficient power to detect common SNPs (MAF = 5% or more) with a relative risk of 1.10 (SI, Table S2).

Last, we cannot exclude that phenotypic heterogeneity influenced our findings. While all cohorts had prospectively-collected information on major health events and diagnoses, heterogeneity in the methods of assessment and classification might have limited the ability to identify true effects.

Complex diseases may result from the effects of a large number of low frequency variants, with substantial allelic heterogeneity at disease-causing loci (40-42). Theoretical modelling that incorporates mutation, random genetic drift, and purifying selection suggests that many of the variants that affect complex traits may be in the 1-5% frequency range (40). Indeed, sequencing of candidate genes in an attempt to capture such low frequency variants, has led to the identification of rare variants with modest effects on body mass index (43-45), triglyceride levels (46), HDL- (46-47) and LDL-cholesterol levels (48-50).

It is impossible to determine the functional variant of a gene by GWAS. Moreover, we cannot conclude from the location of a SNP that this variation is involved in the expression of the closest gene. However, our top results suggested a possible role of genes involved in neurological processes in human longevity and aging. Ten of the 22 suggestive associations identified in our analyses are in or near genes that are highly expressed in the brain (*HECW2*(51), *HIP1*(52), *BIN2*, *GRIA1*), were previously related to the regulation of neuronal excitability and plasticity (*KCNQ4*(53), *LMO4*(54-55), *GRIA1*), and the maintenance of neural circuitry and synaptic plasticity(*NETO1*), or are associated with neurological diseases such as Alzheimer's disease (*LMO4*(55), *BIN2, GRIA1, GRIN2B)* and amyotrophic lateral sclerosis (*GRIN2B*). In addition, 6 of the 22 SNPs were in close proximity to genes associated with other phenotypes of aging such as autophagy (*ATG4C*(6)), cancer (*ATG4C*(56), *HIP1*(57), *HECW2*(51), *VWA5A*(58), *MECOM*), and mitochondrial depletion syndrome (*SUCLA2*). Notably, *BIN2*, *ATG4C*, *KCNQ4*, *MECOM* and *SUCLA2* showed associations with both traits in our study.

Using the expression quantitative trait loci (eQTL) browser (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/) we detected eQTL associated with HIP1, COL5A1, LOC340156, and SMARCA2 in time to death only.

Interestingly, SNPs known to be associated with longevity and disease in the neighbourhood of *APOE*(11) or *FOXO3A*(14-15) only reached nominal significance (results not shown). These genes were originally identified in studies of centenarians; it is possible that our study of cohorts comprised of individuals from the general populations did not have sufficient statistical power to identify these genes with certainty.(59)

While meta-analysis of GWAS has the power to detect small changes of allele frequencies between groups with the analyzed trait, true association signals may not be revealed based on a stringent genome-wide significance threshold. This situation, although limiting false positive findings, performs poorly in identifying false negatives as they may fall below the threshold. Network analyses using a less stringent significance threshold do not amend the overall negative finding of this study. However, it is well-recognized that within the many associations that failed to attain this level of significance lie true positive associations. Network analyses can provide useful information exploring multiple gene effects and their interactions.

In fact the interpretation of most GWAS results is difficult because individual results may involve many seemingly unrelated genes. Since PANTHER and IPA are built on different conceptual approaches, database sources and different pathway classifications, they can be seen as complementary approaches. Our pathway and network analyses highlighted neuronal activities and organism developmental processes as major biological processes involved aging. In addition, it highlighted Wnt signalling and showed that those genes that were involved in most pathways indeed had substantial effects within the analyzed trait. *NTRK2(60)*, *NCAM1*(61), *GRID2*(62), and *RIMS1*(63-64) are associated with neuronal development and disease pathways that were highlighted in the analysis of time to death. *MYC*(65-66), E2F1(67), *EGFR*(68), and *CEBPA*(69-70) are associated with "cancer", "cell function" and "development" pathways.

Few if any of the top hits from the GWAS were involved in common pathways of aging, typically addressed in candidate gene studies. For example, there was no specific evidence for genes involved in IGF-insulin signalling. However, this negative finding cannot be interpreted as evidence against the importance of IGF-insulin signalling, as well as other processes such as inflammation, oxidative stress, cellular damage and repair, growth hormone, and cell proliferation in aging. Moreover, it is possible that polymorphisms in related genes have an effect in the oldest old, who were represented by fewer numbers in our study population such that our study design would be underpowered to detect it. It is also conceivable that the neurological pathways identified by our analysis interact with the known candidate genes involved in aging (20, 71). It is feasible that the traditional aging pathways are hierarchically controlled by neurons and that the brain might be the location coordinating physiological changes (20, 71). Because neurons are particularly susceptible to damage caused by reactive oxygen species, limitations in cellular maintenance and repair might reinforce these pathways and accelerate aging (20). An increased ability of neuronal cells to

prevent or repair oxidative damage might result in beneficial hormonal signalling, otherwise deregulated with age, thus delaying the onset of age-related disease and directly regulating cognitive aging and life span (71-73).

In conclusion, our analysis did provide suggestive evidence that aging is under neuronal control. Unfortunately, we have no relevant tissue or expression experiment available to further underscore or validate our findings. Future investigations of changes of gene expression with age at cellular and population levels are warranted.

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Figure 1a Quantile–quantile (Q-Q) plot after meta-analysis for time to death

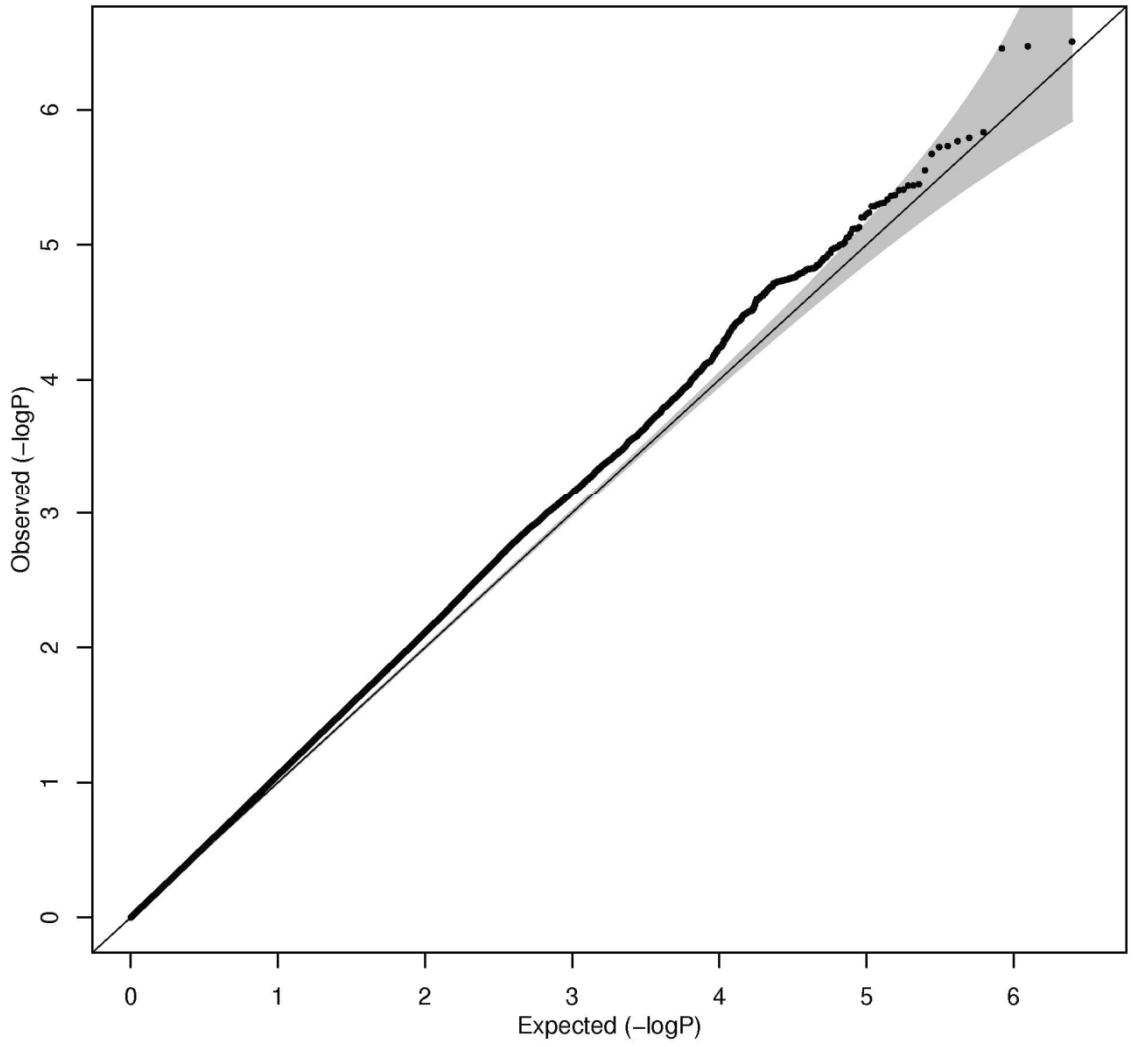


Figure 1b Quantile–quantile (Q-Q) plot after meta-analysis for time to event

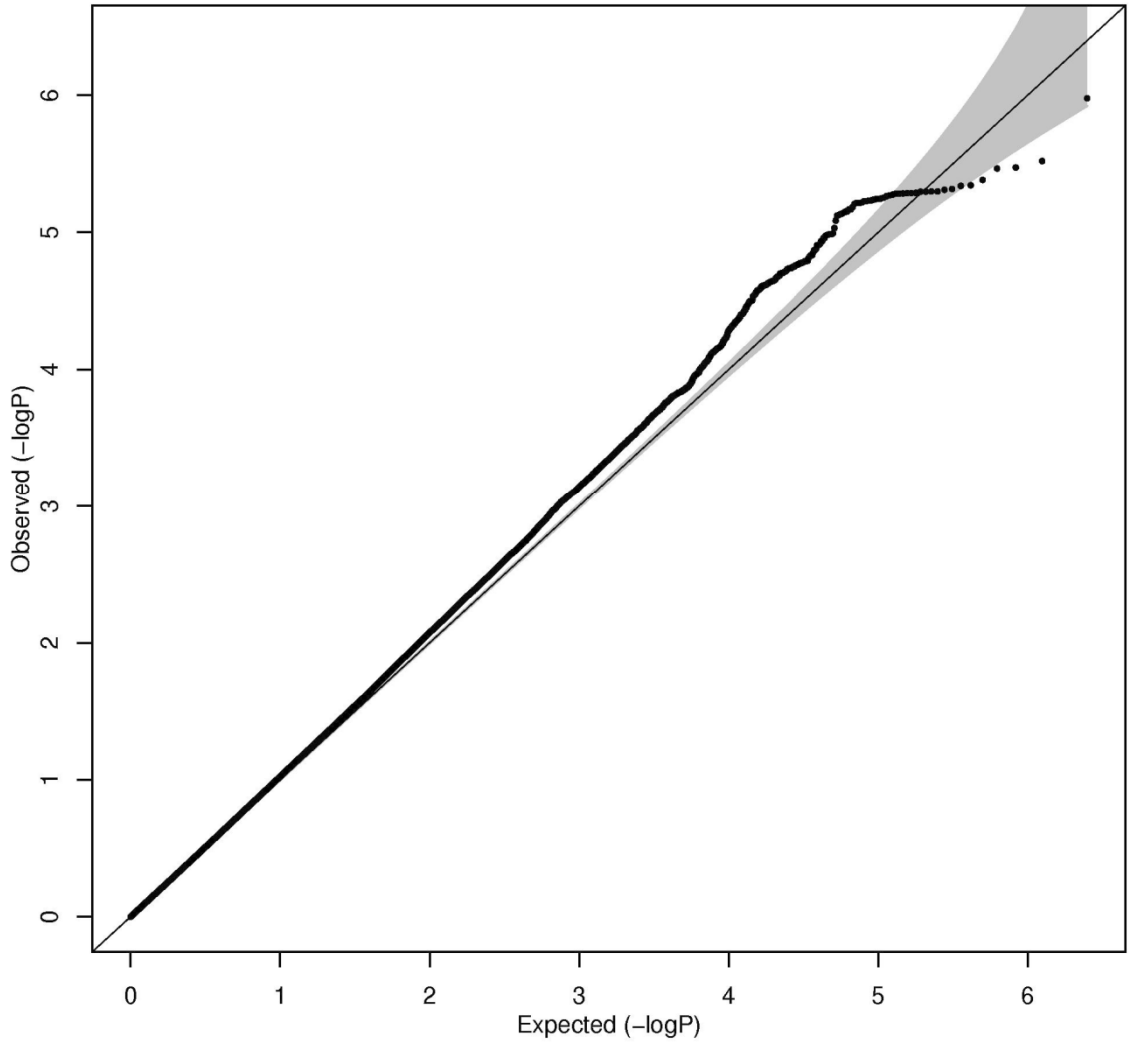
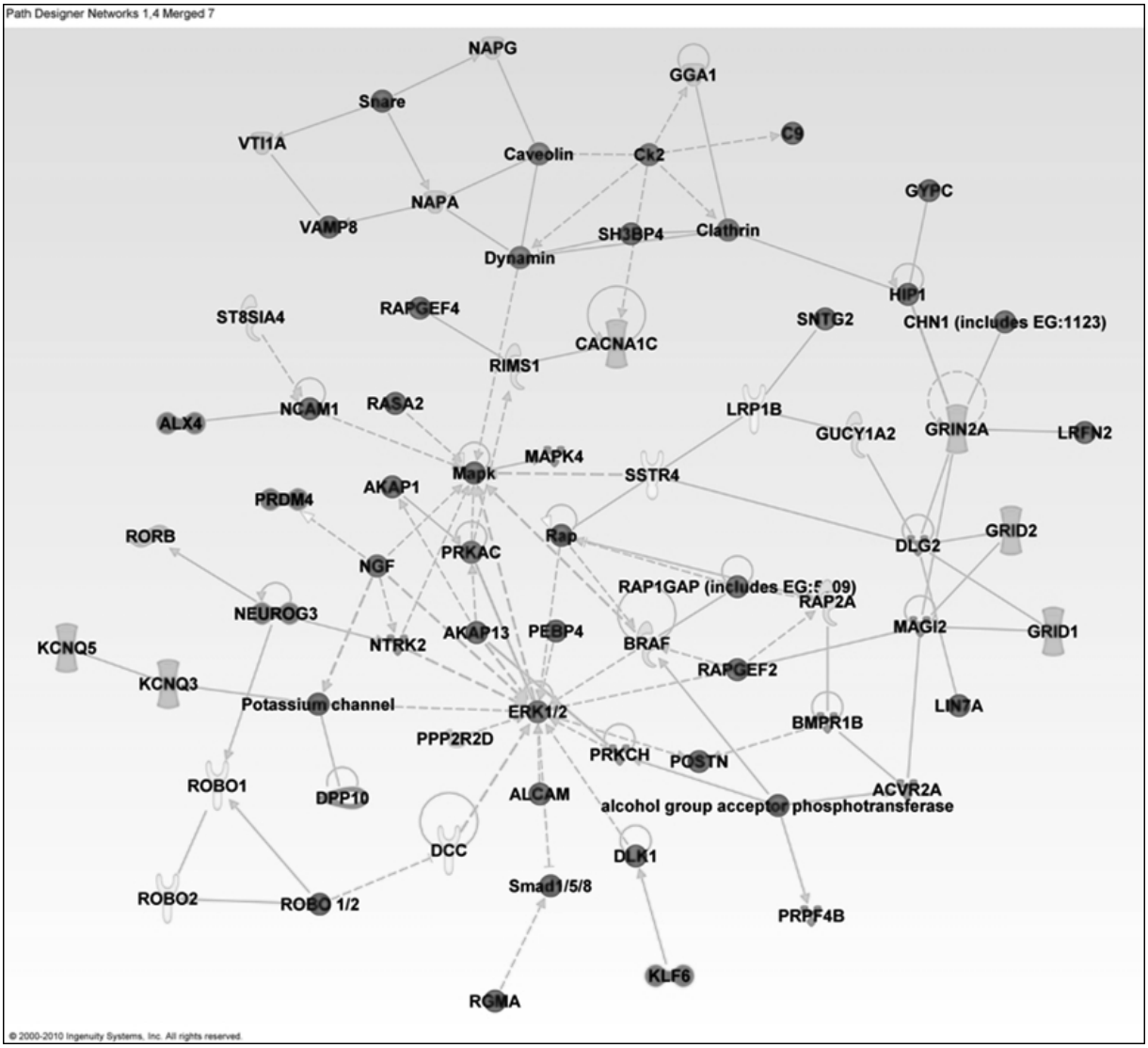


Figure 2 - Network describing neuronal activities related to time to death



Legend: Pathway analysis of genes (SNPs) associated with time to death. Genes are represented as nodes; edges indicate known interactions (solid lines depict direct and hatched lines depict indirect interaction). Human gene functions are color-coded as follows: Red= Unknown, Yellow= Transmembrane Receptor and G-Protein Coupled Receptor, Magenta (Pink-Purple)= Group/Complex/Other, Bright Green= Ion Channel, Hunter Green (Dark Green) = Peptidase, Navy Blue = transcription regulator, Light Blue=Transporter, Beige= Enzyme, Orange= Kinase, Light green= Cytokine, Light Purple= Phosphate, Gray= Translation Regulator, Olive Green=Ligand-dependent nuclear receptor.

Table 1 - Top 14 SNPs (p-value < 10^{-5}) for time to death ranked by p-value, from meta-analysis of 9 cohorts †

Nr.	SNP	Chr	Position	Closest Reference Gene	Distance (bp) from closest gene	Coded Allele	Non-coded Allele	Frequency coded allele	HR	P-value	Study Effect Direction	Number of Supporting SNPs
1	rs4936894	11	123522703	VWA5A	123	A	G	0.226	1.11	3.38E-07	++++-++-+	224
2	rs1425609	3	164164689	OTOL1	1460265	A	G	0.381	0.92	1.46E-06	---------	399
3	rs766903	12	49990101	BIN2	14104	A	G	0.834	0.90	1.61E-06	----+----	7
4	rs12042640	1	63139384	ATG4C	36747	T	C	0.284	1.09	1.71E-06	++++-+-+-	19
5	rs17149227	7	75073485	HIP1	72141	T	G	0.959	0.79	3.56E-06	-??---+-?	0
6	rs3128591	9	136741940	COL5A1	68468	A	G	0.754	0.92	3.64E-06	---------	20
7	rs11582903	1	87618642	LMO4	34804	A	C	0.150	1.12	3.94E-06	++-++++++	38
8	rs4850695	2	196861504	HECW2	89283	A	G	0.766	1.09	4.62E-06	+++++++++	95
9	rs10259086	7	103680248	ORC5L	44549	T	G	0.686	1.08	5.16E-06	++++++-++	72
10	rs2769255	1	41017941	KCNQ4	4329	T	C	0.374	1.08	5.17E-06	++++++-++	95
11	rs17291546	6	2660681	LOC340156	35472	A	G	0.957	0.82	7.65E-06	-?-------	8
12	rs12606100	18	69102967	NETO1	417177	T	C	0.202	1.11	8.72E-06	+??-++++-	4
13	rs1274214	11	122979741	GRAMD1B	18987	T	C	0.500	0.93	8.87E-06	---------	42
14	rs10811679	9	2224701	SMARCA2	41080	T	C	0.330	1.08	9.53E-06	+++++++++	37

N = 25,007 participants with 8,444 deaths, only SNPs with MAF > 3% presented, p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36. Chr = chromosome, Hazard Ratios (HR) are for each additional coded allele. Number of supporting SNPs: the number of SNPs within 500kb of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 (r^2>=0.10) and have association p-value<0.05. Study Effect Direction: study-specific information is presented in the order: RS, CHS, FHS, ARIC, AGES, HABC, BLSA, InCHIANTI, SHIP. Direction: "+" = coded allele increases risk of mortality, "-" = coded allele decreases risk of mortality, ?= not tested. † For information on all SNP associations with p-value<10^{-4} see Table S2

Table 2 - Top 8 SNP (p-value < 10^{-5}) associations from meta-analysis of 8 cohorts for time to event, ranked by p-value (n = 16,995 with 7,314 events)

Nr.	SNP	Chr	Position	Closests Reference Gene	Distance (bp) from closest gene	Coded Allele	Non-coded Allele	Frequency coded allele	HR	P-value	Study Effect Direction	Number of Supporting SNPs
1	rs10412199	19	3878771	ATCAY	307	A	G	0.33	0.91	3.02E-06	--?-++--	6
2	rs16852912	3	170169370	MECOM	114610	T	C	0.08	1.18	3.37E-06	++++-+-+	72
3	rs8001976	13	47285723	SUCLA2	129069	T	C	0.44	1.09	3.43E-06	+++-++--	173
4	rs11162963	1	80507169	ELTD1	1262086	T	C	0.63	1.09	4.15E-06	++++++++	40
5	rs4764043	12	14006749	GRIN2B	17570	T	C	0.08	1.17	6.10E-06	++++++++	2
6	rs3112530	5	152619870	GRIA1	230628	A	G	0.08	0.85	6.79E-06	----+---	130
7	rs10202497	2	237935633	COL6A3	38233	A	C	0.14	0.89	8.22E-06	--------	36
8	rs2367725	1	43988415	ST3GAL3	42611	T	C	0.42	1.08	9.31E-06	++++++-+	119

p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36.

Chr=chromosome, Hazard Ratios (HR) are for each additional coded allele. Number of supporting SNPs: the number of SNPs within 500kb of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 (r2>=0.10) and have association p-value<0.05.

Study Effect Direction: study-specific information is presented in the order: RS, CHS, FHS, ARIC, AGES, HABC, BLSA, InCHIANTI

Direction: + = coded allele increases risk of event, - = coded allele decreases risk of event, ?= not tested

† For information on all SNP associations with p-value<10^{-4} see Supplementary Information, Table S11

Table 3 Overlap between the associations of time to death and time to event*

					time to death		time to event		Top SNPs from time to death (time to event) analysis associated with different p-values in time to event (time to death) analysis					
	Top Hit	SNP	Chr	Closest Reference Gene	P	Effect	P	Effect	TOTAL	P >=0.05	P<0.05	P<0.01	P<0.001	P<0.0001
Time to death														
	1	rs1425609	3	OTOL1	1.46E-06	-	0.005704	-	1119	693	235	132	37	22
	2	rs766903	12	BIN2	1.61E-06	-	0.01315	-	37	27	4	5	0	1
	3	rs12042640	1	ATG4C	1.71E-06	+	0.03701	+	93	60	19	4	0	10
	4	rs11582903	1	LMO4	3.94E-06	+	0.7336	-	133	91	8	12	21	1
	5	rs10259086	7	ORC5L	5.16E-06	+	0.03266	+	239	154	56	21	4	4
	6	rs2769255	1	KCNQ4	5.17E-06	+	0.01322	+	287	151	68	56	7	5
	7	rs17291546	6	LOC340156	7.65E-06	-	0.01624	-	29	19	9	1	0	0
	8	rs12606100	18	NETO1	8.72E-06	+	0.02853	+	23	16	5	2	0	0
	9	rs1274214	11	GRAMD1B	8.87E-06	-	0.0567	-	101	39	28	17	17	0
Time to event														
	1	rs16852912	3	MECOM	0.00589	+	3.37E-06	+	169	67	49	49	2	2
	2	rs8001976	13	SUCLA2	0.01473	+	3.43E-06	+	433	198	91	46	59	39
	3	rs4764043	12	GRIN2B	0.0017	+	6.10E-06	+	45	42	2	1	0	0
	4	rs10202497	2	COL6A3	0.00035	-	8.22E-06	+	135	83	27	12	9	4
	5	rs2367725	1	ST3GAL3	0.0274	+	9.31E-06	+	459	317	56	37	31	18

P: p-values are for the inverse variance-weighted meta-analysis. Chr: chromosome, Effect = meta-analysis direction of effect. Total: the number of SNPs in time to death (time to event) analysis within 500kb of SNPs from the time to event (time to death) analysis that are in LD with the top SNPs from the time to death (time to event) analysis in the HapMap CEU release 22 (r2>=0.10) and have association p-value<0.05.

* only SNPs that were nominally significant (p<0.05) for both traits are shown.

Table 4 – Results from the gene annotation analysis using PANTHER

Biological Process	H. sapiens (Reference)	Nr genes observed	Nr genes expected	-/+	p-value unadjusted	p-value adjusted*
Time to Death:						
Biological process unclassified	11321	238	367,71	-	1,29E-20	4,00E-19
Developmental processes	2152	152	69,9	+	1,39E-19	4,32E-18
Neuronal activities	569	65	18,48	+	8,94E-18	2,77E-16
Signal transduction	3406	199	110,63	+	9,09E-17	2,82E-15
Neurogenesis	587	64	19,07	+	1,43E-16	2,84E-14
Ectoderm development	692	68	22,48	+	2,33E-15	3,38E-13
Cell adhesion	622	57	20,2	+	7,00E-12	2,17E-10
Time to Event:						
Developmental processes	2152	115	57,46	+	1,02E-12	3,16E-11
Biological process unclassified	11321	214	302,27	-	2,93E-12	9,08E-11
Neuronal activities	569	47	15,19	+	2,28E-11	7,08E-10

Legend: Candidate genes (genes observed) were in the neighbourhood of SNPs associated with p-value < 1 x 10^{-3}. For time to death 862 candidate genes were identified; 826 could be matched to the PANTHER gene list. For time to event 704 candidate genes were identified; 679 could be matched to the PANTHER gene list. Extended lists of PANTHER pathways, biological processes, and molecular functions are listed in the Supplementary Information (S12, S13).

* Bonferroni correction multiplying the single-test P-value by the number of independent tests to obtain an expected error rate.

Supplementary information

Study population

Rotterdam Study (RS)

From 1991 to 1995 all inhabitants of Ommoord, a district of Rotterdam, The Netherlands, who were 55 years or older, were invited to participate in the RS (Hofman, et al., 2009,Hofman, et al., 1991). Genotyping information was available for 5,974 participants. All of the participants were followed for incident diseases through linkage to the general practitioner data base and record review by trained medical investigators. General practitioners', hospital records as well as death certificates were used for identification of deaths and health events through 01.01.2009.

Cardiovascular Health Study (CHS)

CHS is a prospective population-based cohort study of CVD, mortality, and other outcomes in 65+ year old Medicare-eligible adults living in four US communities (Fried, et al., 1991). Recruitment of the initial predominantly white cohort was completed in 1990 and 3,267 participants fulfilled the inclusion criteria of this study and had genotyping information available. Only European or European Americans were included in the analysis, so the CHS African Americans were excluded. Major incident health events and deaths were identified through several methods, including: questionnaires completed by participants at each semi-annual contact during follow-up; reports by family members; and periodic searches of the Medicare Utilization database, the National Death Index, and local newspaper obituaries through 30.06.2005. All cardiovascular events were reviewed and adjudicated by an events committee.

Framingham Heart Study (FHS)

FHS was initiated to study determinants of CVD and other major illnesses. The Original cohort was recruited in 1948 and the offspring of the Original cohort participants and offspring spouses were enrolled in 1971 (Dawber, et al., 1963,Dawber, et al., 1951,Feinleib, et al., 1975,Kannel, et al., 1979). DNA was obtained for genetic studies in the 1990s from surviving Original cohort and Offspring participants. The exam at which DNA was obtained is considered the baseline exam for these analyses. For 3,136 participants genetic information was available and the eligibility criteria for this analysis were met. All participants remain

under continuous surveillance and deaths and adjudicated incident diseases that occurred through 01.01.2007 were included.

Atherosclerosis Risk Communities Study (ARIC)

ARIC, initiated in 1987-89, is a population-based cohort study of cardiovascular disease and its risk factors sponsored by the National Heart, Lung and Blood Institute (NHLBI) (ARIC, 1989). 4,511 participants aged 55 or older fulfilled the inclusion criteria of this study and had genotyping information available. Follow-up for clinical events was obtained through annual surveillance to ascertain vital status as well as cardiovascular events, including hospitalizations and deaths, adjudicated by an Events Committee, and complete until 31.12.2005.

Age, Gene/Environment Susceptibility -Reykjavik Study (AGES)

The AGES Study was initiated to examine potential genetic susceptibility and gene/environment interaction. Between 2002 and 2006, baseline exams were conducted in survivors from the Reykjavik Study. 3,219 AGES-Reykjavik participants were eligible for participation and with available genotype information. Follow-up information was complete till 27.04.2009 via linkage to electronic medical records and vital status registry.

Invecchiare nel Chianti (InCHIANTI)

The InCHIANTI study is a population-based epidemiological study aimed at evaluating the factors that influence mobility in the older population living in the Chianti region in Tuscany, Italy that was initiated in 1998. Presence of chronic diseases was ascertained by a combination of assessments by trained geriatrician, medication use, blood tests, and self-reported physician diagnosis. Death was determined using a death registry. 686 subjects with genotype and mortality and disease data assessed through to 06.03.2006 were used for this analysis.

Baltimore Longitudinal Study of Ageing (BLSA)

The BLSA study is a population-based study aimed to evaluate contributors of healthy aging in the older population residing predominantly in the Baltimore-Washington DC area. The study began in 1958, and follow up data through to 20.12.2004 for 599 subjects was used for this analysis. Participants returned for follow-up visits every 1-2 years where presence of chronic disease was assessed by a physician during the physical exam, self-report or medication use. Death was ascertained through death certificates or report by family members.

The Health, Aging and Body Composition (HABC)

The HABC Study is a prospective cohort study of community-dwelling black and white men and women living in Memphis, TN, or Pittsburgh, PA, aged 70-79 years at recruitment in 1997. 1,661 white participants were eligible to participate in this analysis and had genotyping information available. Surveillance was conducted by in person examination alternating with a telephone interview every 6 months. Hospital records, death certificates, and autopsy data were reviewed by committee to adjudicate causes of death and complete through 26.11.2007.

Study of Health in Pomerania (SHIP)

SHIP is a cross-sectional survey in West Pomerania, the north-east area of Germany, initiated in 1997 (John, et al., 2001,Volzke, et al., 2010). 1717 participants were eligible to participate in this study and had genotyping information available. Information on vital status was acquired at annual intervals from the local health authority and complete through 03.11.2009.

Figure S1 Genome-wide signal intensity plot of association by chromosome for time to death

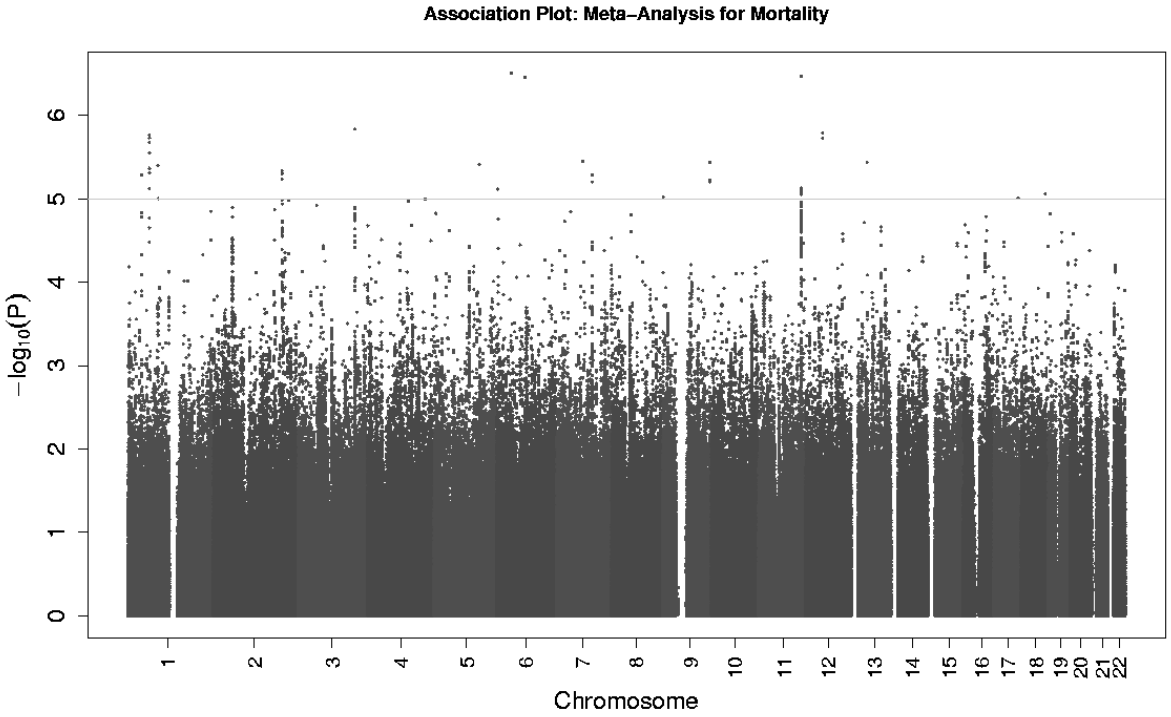


Figure S2 Genome-wide signal intensity plot of association by chromosome for time to event

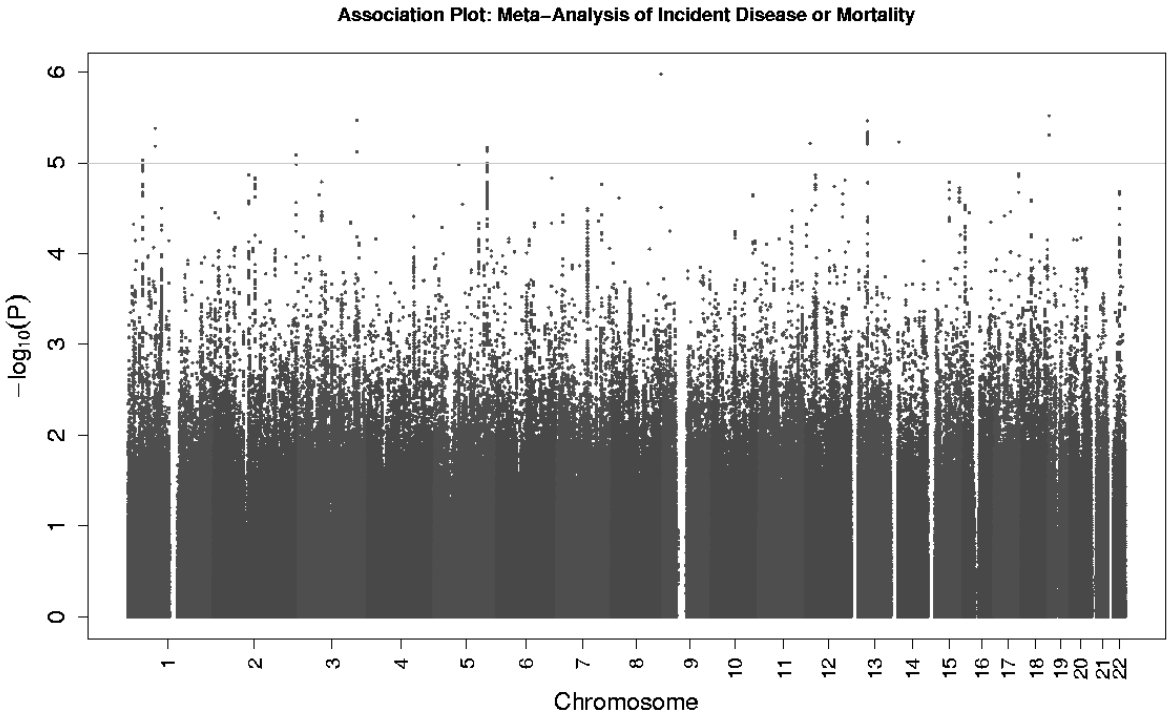
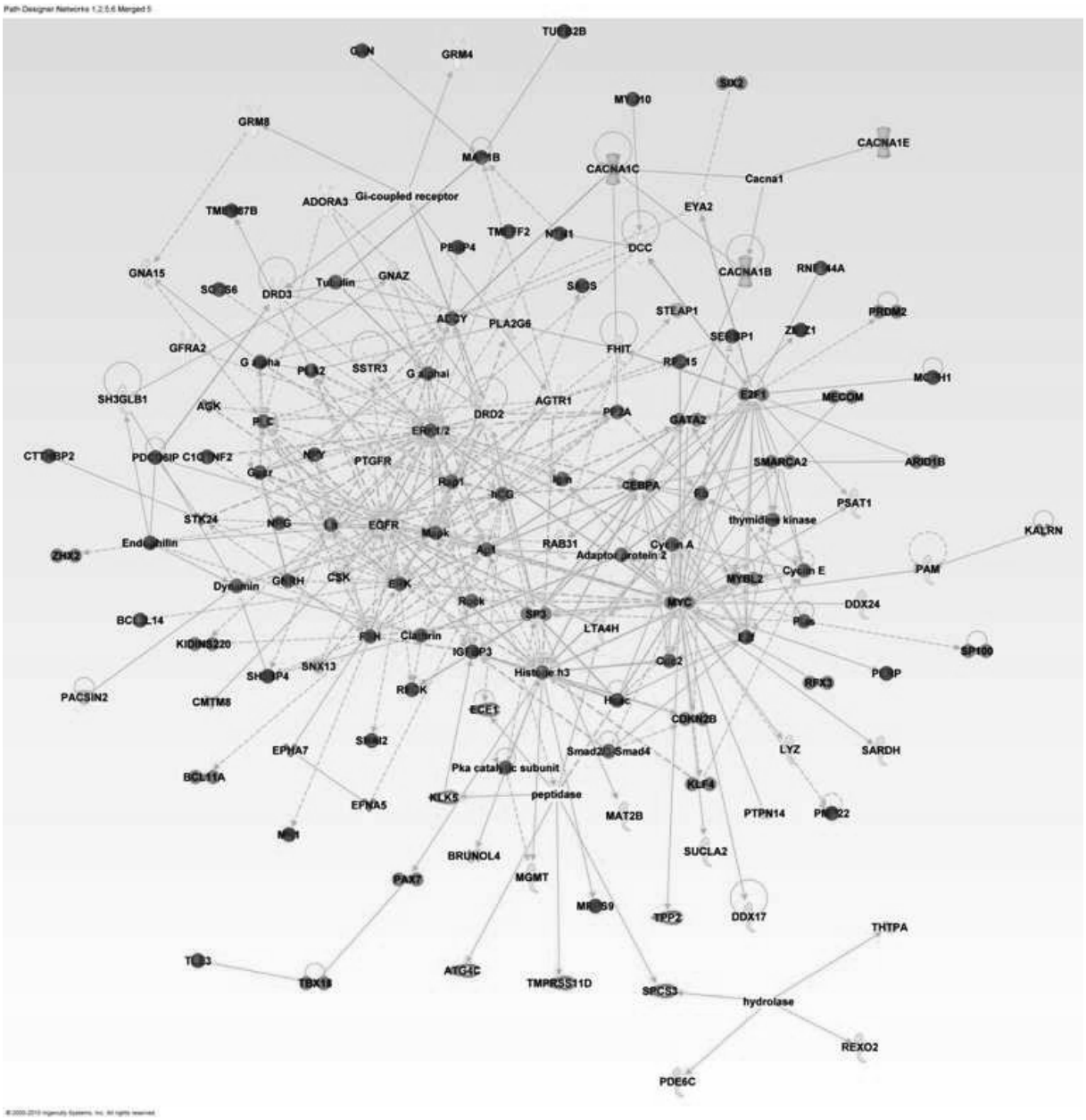


Figure S3 Network describing cellular function and development related to time to event



Legend: Pathway analysis of genes (SNPs) associated with time to event. Genes are represented as nodes; edges indicate known interactions (sold lines depict direct and hatched lines depict indirect interaction). Human gene functions are color-coded as follows: Red= Unknown, Yellow= Transmembrane Receptor and G-Protein Coupled Receptor, Magenta (Pink-Purple)= Group/Complex/Other, Bright Green= Ion Channel, Hunter Green (Dark Green) = Peptidase, Navy Blue = transcription regulator, Light Blue=Transporter, Beige= Enzyme, Orange= Kinase, Light green= Cytokine, Light Purple= Phosphate, Gray= Translation Regulator, Olive Green=Ligand-dependent nuclear receptor.

Table S1 Information on genotyping, quality control and imputation by cohort

Study	AGES	CHS	FHS	RS
Array type	Illumina 370CNV	Illumina 370CNV	Affymetrix 500K and MIPS 50K combined	Version 3 Illumina Infinium II HumanHap550 SNP chip array
Genotyping center	NIA Laboratory of Neurogenetics	Cedars-Sinai Medical Center	Affymetrix Core Laboratory	Erasmus Medical Center
Genotype calling	Illumina BeadStudio	Illumina BeadStudio	BRLMM	Illumina Beadstudio
Exclusion on SNPs used for imputation	Call rate <97%, HWE $p<1^{-6}$, MAF <1%, Mishap $p<1^{-9}$, A/T and G/C SNPs, Mismatches between Illumina, dbSNP and/or HapMap position	Call rate≤97%, HWE $p<10^{-5}$, >2 duplicate errors or Mendelian inconsistencies (for reference CEPH trios) heterozygote frequency=0, not in HapMap	Call rate <97%, HWE $p<1^{-6}$, Mishap $p<1^{-9}$, Mendelian errors >100	Call rate <90%, No MAF/HWE filter
Exclusion on a per sample basis	Sex mismatch, Sample failure, Genotype mismatch with reference panel	Call rate <95%, sex mismatch, sample failure	Call rate <97%, subject heterozygosity >5 SD away from the mean, large Mendelian error rate	Call rate <97.5% sex mismatch, excess autosomal heterozygosity >0.336, outliers identified by the IBS clustering analysis
Imputation	MACH (version 1.00.16	BIMBAM10 v0.99	MACH (version 1.00.15)	Mach 1.0
Imputation Backbone (NCBI build)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)
Data handling and statistical tests	PLINK and R	R	R packages kinship, GEE, COXPH	PLINK, ProbABLE, Mach2QTL,

Study	ARIC	HABC	InCHIANTI	BLSA	SHIP
Array type	Affymetrix GeneChip SNP Array 6.0	Illumina Human1M-Duo	Illumina 550K	Illumina 550K	Affymetrix SNP Array 6.0
Genotyping center	Broad Institute	Center for Inherited Disease Research (NIH)	Laboratory of Neurogenetics (NIA)	Laboratory of Neurogenetics (NIA)	Affymetrix, Inc./Greifswald University
Genotype calling	Birdseed	Illumina BeadStudio	BeadStudio	BeadStudio	Birdseed V2
Exclusion on SNPs used for imputation		call rate < 97%, HWE p<10-6, MAF <1%,	call rate < 99%, HWE p<10-4, MAF <1%,	call rate < 99%, HWE p<10-4, MAF <1%,	NA
Exclusion on a per sample basis	sample failure, genotypic sex mismatch, and first-degree relative,	sample failure, genotypic sex mismatch, and first-degree relative in samples set	Call rate <97%, heterozygosity < 0.3, sex mismatch	Non-European decent, call rate < 98.5%, sex mismatch, duplication	Call rate <92%, IBS duplicates, sex mismatch
Imputation	MACH v1.0.16	MACH (version 1.0.16)	MACH (version 1.0.16)	MACH (version 1.0.16)	IMPUTE v0.5.0
Imputation Backbone (NCBI build)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36))
Data handling and statistical tests	ProbABEL	R	ProbABEL, R, Perl	ProbABEL, R, Perl	ProbABEL, InforSense, Caché

Table S2 Power analysis for time to death

With 8,444 events, 80% Power, and two sided alpha = 0.05 a HR of 1.25 at MAF = 1% is detectable.

Minor Allele Frequency	Hazard Ratio
0.01	1.25
0.02	1.20
0.03	1.15
0.04	1.12
0.05	1.10
0.1	1.09
0.15	1.07
0.2	1.07
0.25	1.06
0.3	1.05
0.35	1.05
0.4	1.05
0.45	1.05
0.5	1.05

The table is similar for time to event with 7,314 events but the detectable HR at a specific MAF is higher.

Table S3 Descriptive statistics of 9 cohorts participating in the analysis of time to death

Study	N	N deceased	Mean age at Baseline (±SD)	Mean age at death (±SD)	Sex, % female	Mean follow-up time in years (±SD)
Rotterdam Study (RS)	5974	3174	69.4 (9.1)	83.2(8.3)	59%	12.5 (5.2)
Cardiovascular Health Study (CHS)	3267	1718	72.3 (5.4)	83.4 (6.3)	61%	12.3 (4.2)
Framingham Heart Study (FHS)	3136	654	70.0 (10.2)	83.0 (9.3)	56%	6.0 (2.4)
Atherosclerosis Risk Communities Study (ARIC)	4511	1108	59.4 (2.9)	71.3 (5.4)	50%	15.7 (3.7)
Age, Gene/Environment Susceptibility -Reykjavik Study (AGES)	3219	558	76.4 (5.5)	79.3 (5.9)	58%	5.2(1.3)
Invecchiare nel Chianti (InCHIANTI)	902	183	72.5 (7.7)	85.4 (7.9)	56%	5.9 (0.9)
Baltimore Longitudinal Study of Ageing (BLSA)	620	183	62.0 (8.8)	86.8 (8.0)	41%	15.7 (8.2)
The Health, Aging and Body Composition (HABC)	1661	460	73.8 (2.8)	80.4 (3.7)	47%	8.2 (2.3)
Study of Health in Pomerania (SHIP)	1717	406	66.4 (7.2)	76.9 (7.2)	47%	9.2 (2.4)
TOTAL	25007	8444	69.0 (8.9)	81.1 (8.4)	55%	10.6 (5.4)

Table S4 Extended table of association results for the analysis of time to death with $p < 1 \times 10^{-4}$*

Nr.	SNP	Chr	Position	Closests Reference Gene	Distance (bp) from closest gene	Coded Allele	Non-coded Allele	Frequency coded allele	HR	P-value	Study Effect Direction	Number of Supporting SNPs
1	rs4936894	11	123522703	VWA5A	123	A	G	0.2256	1.1074	3.38E-07	++++-++-+	224
2	rs1425609	3	164164689	OTOL1	1460265	A	G	0.3813	0.9223	1.46E-06	---------	399
3	rs766903	12	49990101	BIN2	14104	A	G	0.8342	0.9047	1.61E-06	----+----	7
4	rs12042640	1	63139384	ATG4C	36747	T	C	0.284	1.0861	1.71E-06	++++-+-+-	19
5	rs17149227	7	75073485	HIP1	72141	T	G	0.9587	0.7889	3.56E-06	-??---+-?	0
6	rs3128591	9	136741940	COL5A1	68468	A	G	0.7543	0.9206	3.64E-06	---------	20
7	rs11582903	1	87618642	LMO4	34804	A	C	0.15	1.1162	3.94E-06	++-++++++	38
8	rs4850695	2	196861504	HECW2	89283	A	G	0.7659	1.0885	4.62E-06	+++++++++	95
9	rs10259086	7	103680248	ORC5L	44549	T	G	0.6862	1.0823	5.16E-06	++++++-++	72
10	rs2769255	1	41017941	KCNQ4	4329	T	C	0.374	1.079	5.17E-06	++++++-++	95
11	rs17291546	6	2660681	LOC340156	35472	A	G	0.9569	0.8173	7.65E-06	-?-------	8
12	rs12606100	18	69102967	NETO1	417177	T	C	0.2019	1.114	8.72E-06	+??-++++-	4
13	rs1274214	11	122979741	GRAMD1B	18987	T	C	0.5002	0.932	8.87E-06	---------	42
14	rs10811679	9	2224701	SMARCA2	41080	T	C	0.3304	1.0825	9.53E-06	+++++++++	37
15	rs13410982	2	216576843	MREG	9748	C	G	0.6241	0.8869	1.05E-05	-?--?----	6
16	rs4600702	2	54452261	FLJ40298	10043	C	G	0.1098	0.8872	1.26E-05	---------	83
17	rs1406752	2	175606663	CHN1	28302	A	G	0.2763	0.9223	1.34E-05	-----+++-	14
18	rs785970	1	239070097	RGS7	64658	A	G	0.7041	1.0839	1.41E-05	++++++-++	26
19	rs16876639	5	6002236	FLJ33360	361317	A	G	0.9642	0.8294	1.48E-05	--+------	6
20	rs1982082	19	6273442	ASAH3	11120	T	C	0.6253	0.9149	1.50E-05	--??----?	1
21	rs9657161	8	55645200	RP1	45979	A	G	0.6721	0.9315	1.55E-05	----+++--	51
22	rs12446736	16	65550194	CES3	2444	T	C	0.9784	0.7651	1.63E-05	-?----+-+	23
23	rs4959896	6	3864713	FAM50B	68164	T	C	0.1644	1.1024	1.75E-05	+++++++++	30
24	rs13075153	3	164355885	LOC131149	1651461	A	T	0.9305	1.2067	1.83E-05	+?++++-++	3
25	rs12673983	7	23620651	CCDC126	17129	A	G	0.8622	0.9062	1.87E-05	--+-+---+	17
26	rs12600138	16	5215419	FAM86A	127637	A	G	0.8631	1.154	2.05E-05	+?+++++++	2
27	rs11725588	4	127263523	FAT4	631151	A	G	0.0522	1.2415	2.08E-05	+?++++-++	49
28	rs4974613	4	1357552	KIAA1530	14180	A	G	0.2932	1.0776	2.11E-05	++-++++++	80
29	rs4550343	13	85479868	SLITRK6	208384	A	G	0.345	0.9262	2.17E-05	---------	142
30	rs9889219	16	68278916	NFAT5	17138	A	G	0.2341	1.0838	2.39E-05	++++++-++	98

31	rs12518724	5	44190859	FGF10	149994	A	G	0.1665	0.9148	2.40E-05	---------	26
32	rs8100126	19	39302181	LSM14A	53129	A	G	0.504	0.9369	2.52E-05	----++---	31
33	rs3743518	16	15867727	C16orf63	650	T	C	0.5954	0.9332	2.52E-05	---------	35
34	rs12425421	12	107847776	SVOP	18990	A	G	0.0425	1.173	2.62E-05	+++++++--	6
35	rs17446441	20	8656166	PLCB1	157379	A	G	0.6756	1.0756	2.64E-05	++-+++++-	70
36	rs2730267	7	158547634	VIPR2	34007	T	C	0.6737	1.0789	2.96E-05	++?++++++	42
37	rs6832976	4	40698328	APBB2	187333	T	C	0.9407	0.8681	3.08E-05	---------	44
38	rs4611001	1	238971251	RGS7	34188	A	G	0.9705	0.8076	3.09E-05	-----+---	2
39	rs17244695	4	182509480	ODZ3	972650	A	G	0.0382	0.7869	3.17E-05	-?---++--	14
40	rs1995100	17	28606834	ACCN1	37285	A	G	0.5017	0.9359	3.31E-05	------+--	45
41	rs7165300	15	83871073	AKAP13	146199	C	G	0.6625	0.9351	3.40E-05	-------+-	171
42	rs9997032	4	94605786	GRID2	306886	A	G	0.665	0.9342	3.46E-05	-----+---	64
43	rs4710604	6	67206700	EGFL11	860282	A	C	0.2486	1.1077	3.56E-05	+?++++++-	33
44	rs6808405	3	72078018	PROK2	161116	A	C	0.2654	1.0883	3.66E-05	+?++-+-+-	26
45	rs7187274	16	8530449	C16orf68	92578	A	T	0.1301	1.1006	3.69E-05	+++++++++	59
46	rs1382652	5	101060207	SLCO4C1	537383	A	G	0.741	1.0766	3.70E-05	++-++--++	176
47	rs4807137	19	1696610	ONECUT3	8051	A	G	0.7558	0.9175	3.73E-05	--??----?	4
48	rs1727504	2	54281638	TSPYL6	52180	T	C	0.532	0.9362	3.76E-05	---------	39
49	rs17531288	20	56152764	C20orf85	6624	T	C	0.0769	0.8501	4.16E-05	---------	3
50	rs999228	7	138051398	ATP6V0A4	9819	A	C	0.5389	0.937	4.17E-05	------++-	6
51	rs7580648	2	205376297	PARD3B	257537	A	G	0.11	0.9007	4.39E-05	-----+--+	63
52	rs10500512	16	63767040	CDH11	53620	A	G	0.79	0.9257	4.59E-05	------+--	18
53	rs1383756	1	216939247	TGFB2	257654	T	C	0.1237	1.1333	4.71E-05	+?++++--+	40
54	rs1530609	4	5214348	STK32B	109921	A	G	0.4593	0.9352	4.82E-05	---------	19
55	rs2295527	14	90732368	C14orf159	29088	A	G	0.9033	1.1159	4.93E-05	++-+++-++	4
56	rs17799174	8	73052557	TRPA1	43483	A	T	0.8714	1.1078	5.00E-05	+++++-+++	10
57	rs1327474	6	137582768	IFNGR1	568	T	C	0.5416	1.0702	5.40E-05	+++++++++	23
58	rs6075131	20	16790276	OTOR	109468	A	G	0.048	1.175	5.41E-05	+?+++-+++	5
59	rs11028131	11	24716677	LUZP2	241546	A	G	0.1595	1.109	5.61E-05	+?+-+++-+	10
60	rs935526	3	77642707	ROBO2	136644	T	C	0.6494	0.935	5.63E-05	---+-+---	74
61	rs16878625	8	89195106	MMP16	44530	T	C	0.9212	0.8867	5.79E-05	---------	54
62	rs2708754	19	58717784	ZNF331	1796	A	G	0.3847	0.9368	5.79E-05	-----+---	17
63	rs2687381	4	32960156	PCDH7	2202637	T	C	0.0351	1.2437	5.83E-05	+++++++-+	106
64	rs2160591	12	106661655	PRDM4	10882	T	C	0.2284	0.9243	6.13E-05	----+----	30
65	rs2807305	9	81367110	TLE4	9587	T	C	0.1051	0.8999	6.17E-05	-------++	57

66	rs9456882	6	158277849	SNX9	8248	A	T	0.9818	0.7216	6.25E-05	-??---+--	7
67	rs5996347	22	21361721	GGTL4	41353	T	C	0.1247	0.9066	6.28E-05	----+---+	7
68	rs7197382	16	74107386	CHST5	12544	A	C	0.75	1.0773	6.37E-05	+-+-++++-	60
69	rs790669	5	114813364	FEM1C	71142	T	C	0.4435	0.9388	6.45E-05	---------	36
70	rs12409187	1	4633653	AJAP1	18689	A	G	0.0463	1.1652	6.54E-05	+++++++--	16
71	rs12777662	10	127240024	C10orf137	158049	A	G	0.9362	0.8738	6.69E-05	--+------	5
72	rs11107420	12	93078944	PLXNC1	12315	A	G	0.1459	1.091	6.80E-05	++++-+--+	22
73	rs9554503	13	98221565	SLC15A1	18656	A	T	0.7137	1.0724	6.98E-05	++++++-++	132
74	rs17437544	7	27197714	HOXA13	5309	T	C	0.967	0.6691	7.24E-05	-???---??	1
75	rs17831313	14	51346712	GNG2	50087	C	G	0.8155	0.9196	7.31E-05	----+-+--	33
76	rs12040905	1	119654754	HAO2	58170	C	G	0.9125	0.8948	7.36E-05	---+-+---	21
77	rs438129	3	11982910	SYN2	37951	T	C	0.2161	1.0776	7.40E-05	+++++++--	25
78	rs7699752	4	113634851	NEUROG2	19271	T	C	0.9416	1.1582	7.55E-05	++++++-++	15
79	rs10211241	2	200275381	FLJ38973	208892	T	C	0.6423	1.066	7.62E-05	++++++-++	65
80	rs9646894	2	123111902	TSN	870006	A	G	0.9842	0.7326	7.64E-05	-??+--+?-	2
81	rs10124818	9	1453618	DMRT2	406066	A	G	0.8198	0.9201	7.73E-05	---------	27
82	rs12416320	10	70818734	HK1	12907	A	G	0.0639	0.8454	7.85E-05	-?-------	9
83	rs1072975	2	190829427	HIBCH	51823	A	T	0.8313	0.9148	8.18E-05	-----+-+-	31
84	rs9567731	13	46298687	HTR2A	6826	A	G	0.2618	0.9179	8.20E-05	------+--	42
85	rs41026	7	103927757	LHFPL3	171418	T	G	0.9412	0.855	8.36E-05	-?----+--	29
86	rs205204	6	89781137	RNGTT	51070	T	C	0.5695	1.0657	8.45E-05	+++++++++	43
87	rs2148294	10	6298058	PFKFB3	13158	A	G	0.9521	0.8496	8.56E-05	--??--+-?	0
88	rs6540118	16	86538260	BANP	4278	T	C	0.2432	1.0761	8.57E-05	++++++--+	4
89	rs12548355	8	22745696	PEBP4	95670	A	T	0.6009	1.0646	8.64E-05	+++++-+-+	29
90	rs9464371	6	56260902	COL21A1	40565	A	G	0.3155	0.9346	8.73E-05	--+-----+	28
91	rs9383834	6	150998910	PLEKHG1	36219	T	C	0.7862	0.9206	8.81E-05	-++------	23
92	rs9811637	3	164724724	SI	1454655	T	G	0.6297	0.9358	8.83E-05	---------	66
93	rs317415	17	29205289	ACCN1	302649	T	C	0.8976	0.9015	8.85E-05	-------+-	21
94	rs519067	9	76513592	TRPM6	13638	T	G	0.4493	1.064	8.90E-05	++++-+--+	3
95	rs7304170	12	27897108	KLHDC5	49869	T	C	0.1555	0.9134	9.15E-05	------+++	7
96	rs13132542	4	11142521	HS3ST1	102886	T	G	0.4133	1.0668	9.19E-05	+++++++-+	11
97	rs11222479	11	130506744	SNX19	215152	A	G	0.1007	1.1034	9.36E-05	+++++++++	12
98	rs13083177	3	163769051	LOC131149	1064627	T	C	0.9327	1.1387	9.62E-05	++++--+++	37
99	rs1489333	1	162950362	PBX1	132571	A	G	0.949	1.1997	9.73E-05	+?+++++-+	21
100	rs13182040	5	171719466	SH3PXD2B	26357	T	G	0.2856	0.9329	9.75E-05	--+---+-+	36

101	rs2129107	9	83539441	TLE1	46025	T	C	0.3697	1.0662	9.99E-05	+++++++-+		30

p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36.
Chr=chromosome, Hazard Ratios (HR) are for each additional coded allele
Number of supporting SNPs: the number of SNPs within 500kb of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 (r2>=0.10) and have association p-value<0.05.
Study Effect Direction: study-specific information is presented in the order: RS, CHS, FHS, ARIC, AGES, HABC, BLSA, InCHIANTI, SHIP
Direction:+ = coded allele increases risk of mortality, - = coded allele decreases risk of mortality, ?= not tested
*SNPs with a MAF < 3% are not displayed

Table S5 Replication Studies

Replication Study	Study Description
Whitehall II	The Whitehall II Study recruited 10,308 participants (70% men) between 1985 and 1989 and involved 20 London based civil service departments. DNA was stored from phase 7 from over 6,000 participants aged between 50 and 75.Analyses here are restricted to those aged 55 and over. The study individuals are all highly phenotyped for cardiovascular and other ageing related health outcomes. Nearly all participants (99%) are also consented to linkage to routine data such as mortality registers and hospital episode statististics, which allows for the assessment of health outcomes and mortality. Mortality data are available to January 2010. New genotyping was conducted using Kaspar technology (KBiosciences, Hoddeston. UK). Duplicates were examined for 5% of samples and were found to be less than 0.2% [Marmot and Brunner, Int J Epidemiol. 2005;34(2):251-6.]
ELSA	English Longitudinal Study of Ageing (ELSA) is a national cohort of participants (48% men) aged over 50 years recruited from the Health Surveys for England in 1998, 1999 and 2001. Genetic data were collected at wave 2 of the study (2004/5). A wide range of phenotypic measures relevant to ageing are available. Nearly all participants (97%) are also consented to linkage to routine data such as mortality registers and hospital episode statististics, which allows for the assessment of health outcomes and mortality. Mortality data are available to January 2010. New genotyping was conducted using Kaspar technology (KBiosciences, Hoddeston. UK). Duplicates were examined for 10% of samples and were found to be less than 0.2%. (http://www.ifs.org.uk/elsa/)
Religious Order Study (ROS)	The Rush Religious Order Study (ROS) enrolled older Catholic nuns, priests, and brothers without known dementia from more than 40 groups across the United States.All participants agreed to annual clinical evaluation and organ donation at the time of death. Details of the clinical evaluation have been previously reported.[1,2] Since January 1994, more than 1,100 participants completed their baseline evaluation. The follow-up rate of survivors exceeds 90%, so does the autopsy rate. In 2008 non-Hispanic white participants were genotyped on the Affymetrix Genechip 6.0 platform at the Broad Institute's Center for Genotyping or the Translational Genomics Research Institute. Genotype information from 810 non-Hispanic whites passed genotyping quality control [Chibnik LB, Shulman JM, Leurgans SE, et al. The Alzheimer's susceptibility locus CR1 is associated with increased amyloid plaque burden and age-related cognitive decline. Annals of Neurology. In press.], of whom 404 have died. The study was approved by the Institutional Review Board of Rush University Medical Center.
Memory and Aging Project (MAP)	The Rush Memory and Aging Project (MAP) enrolled older persons without known dementia from more than 40 retirement communities, senior housing and social service agencies across northeastern Illinois. All participants agreed to annual clinical evaluation and organ donation at the time of death. Details of the clinical evaluation have been previously reported.(Bennett, et al., 2005,Buchman, et al., 2007) Since October 1997, more than 1,300 participants completed their baseline evaluation. The follow-up rate of survivors exceeds 90%, and the autopsy rate exceeds 80%. In 2008 non-Hispanic white participants were genotyped on the Affymetrix Genechip 6.0 platform at the Broad Institute's Center for Genotyping or the Translational Genomics Research Institute. Genotype information from 888 non-Hispanic whites passed genotyping quality control [Chibnik LB, Shulman JM, Leurgans SE, et al. The Alzheimer's susceptibility locus CR1 is associated with increased amyloid plaque burden and age-related cognitive decline. Annals of Neurology. In press.], of whom 346 have died. The study was approved by the Institutional Review Board of Rush University Medical Center.

Table S6 Descriptive statistics of replication samples: time to death

Study	N	N deceased	Mean age at Baseline (±SD)	Mean age at Death (±SD)	Sex, % female	Mean follow-up time in years (±SD)
Whitehall II	3991	151	62.7 (5.2)	68.9 (3.5)	27%	6.3 (0.8)
ELSA	4722	394	67.3 (9.1)	78.4 (9.9)	55%	5.1 (0.9)
ROS	810	404	75.7 (7.4)	87.1 (6.9)	66%	9.6 (4.7)
MAP	888	346	81.1 (6.7)	88.6 (5.7)	72%	6.1 (2.7)
TOTAL	10411	1295	67.4 (9.3)	82.7 (9.9)	47%	6.0 (2.1)

Table S7 Information on genotyping, quality control and imputation by replication study

Study	Whitehall II	ELSA	ROS	MAP
Array type	Kaspar	Kaspar	Affymetrix Genechip 6.0	Affymetrix Genechip 6.0
Genotyping center	KBioscience	Kbioscience	Broad Institute's Center for Genotyping; Translational Genomics Research Institute	Broad Institute's Center for Genotyping; Translational Genomics Research Institute
Genotype calling			Birdsuite Broad Institute	Birdsuite Broad Institute
Exclusion on SNPs used for imputation			HWE p < $1x10^{-6}$; MAF < 0.01, genotype call rate < 0.95; misshap test < $1x10^{-9}$	HWE p < $1x10^{-6}$; MAF < 0.01, genotype call rate < 0.95; misshap test < $1x10^{-9}$
Exclusion on a per sample basis			genotype success rate <95%, genotype-derived gender discordant with reported gender, inbreeding coefficient F>0.04	genotype success rate <95%, genotype-derived gender discordant with reported gender, inbreeding coefficient F>0.04
Imputation			MACH (version 1.0.16a)	MACH (version 1.0.16a)
Imputation Backbone (NCBI build)			HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)
Data handling and statistical tests			PLINK, SAS	PLINK, SAS

Table S8 Replication analysis of 5 SNPs from time to death analysis in 4 independent samples

SNPID	Chr	position	A1	A2	Study	N	Deaths	Frequency (Allele 1)	beta	se	p-value	HR
rs4936894	11	123522703	A	G	RS	5974	3174	0.21	0.10	0.03	6.04E-04	1.11
					CHS	3267	1718	0.21	0.15	0.07	3.18E-02	1.16
					FHS	3136	654	0.24	0.12	0.07	8.75E-02	1.13
					ARIC	4511	1108	0.24	0.06	0.05	2.39E-01	1.06
					AGES	3219	558	0.29	-0.01	0.07	9.38E-01	1.00
					HABC	902	183	0.21	0.21	0.08	6.44E-03	1.24
					BLSA	620	183	0.23	0.05	0.12	6.91E-01	1.05
					InCHIANTI	1661	460	0.19	-0.02	0.14	8.65E-01	0.98
					SHIP	1717	406	0.23	0.20	0.09	1.89E-02	1.22
					Nine study meta-analysis	**25007**	**8444**	**0.23**	**0.10**	**0.02**	**3.38E-07**	**1.11**
					Whitehall II	4002	152	0.23	-0.16	0.15	2.73E-01	0.85
					ELSA	4703	390	0.23	-0.05	0.09	6.03E-01	0.96
					ROS	810	404	0.24	0.09	0.08	2.61E-01	1.10
					MAP	888	346	0.24	0.03	0.09	7.33E-01	1.03
					Meta-analysis of 4 replication studies	**10503**	**1292**	**0.23**	**0.01**	**0.05**	**8.49E-01**	**1.01**
					Thirteen study meta-analysis	**35410**	**9736**	**0.23**	**0.09**	**0.02**	**1.86E-06**	**1.09**
rs1425609	3	164164689	A	G	RS	5974	3174	0.40	-0.08	0.03	3.60E-03	0.93
					CHS	3267	1718	0.36	-0.09	0.04	2.51E-02	0.91
					FHS	3136	654	0.36	-0.14	0.06	2.21E-02	0.87
					ARIC	4511	1108	0.37	-0.04	0.05	4.46E-01	0.97
					AGES	3219	558	0.37	0.00	0.07	9.57E-01	1.00
					HABC	902	183	0.39	-0.08	0.07	2.66E-01	0.92
					BLSA	620	183	0.40	-0.16	0.11	1.44E-01	0.85
					InCHIANTI	1661	460	0.34	-0.25	0.11	2.59E-02	0.78
					SHIP	1717	406	0.39	-0.10	0.08	1.78E-01	0.90
					Nine study meta-analysis	**25007**	**8444**	**0.38**	**-0.08**	**0.02**	**1.46E-06**	**0.92**
					Whitehall II	3978	149	0.37	-0.03	0.12	7.95E-1	0.97
					ELSA	4730	395	0.37	0.03	0.07	7.07E-1	1.03
					ROS	810	404	0.39	-0.06	0.08	4.63E-01	0.95
					MAP	888	346	0.39	-0.12	0.08	1.17E-01	0.88
					Meta-analysis of 4 replication studies	**10506**	**1294**	**0.38**	**-0.04**	**0.04**	**2.74E-01**	**0.96**
					Thirteen study meta-analysis	**35413**	**9738**	**0.38**	**-0.08**	**0.02**	**1.11E-06**	**0.93**

rs766903	12	49990101	A	G	RS	5974	3174	0.84	-0.11	0.03	8.40E-04	0.89
					CHS	3267	1718	0.83	-0.09	0.05	5.18E-02	0.92
					FHS	3136	654	0.83	-0.08	0.08	2.87E-01	0.92
					ARIC	4511	1108	0.84	-0.15	0.06	1.42E-02	0.86
					AGES	3219	558	0.82	0.00	0.08	9.93E-01	1.00
					HABC	902	183	0.82	-0.13	0.09	1.40E-01	0.88
					BLSA	620	183	0.85	-0.12	0.15	4.13E-01	0.88
					InCHIANTI	1661	460	0.86	-0.01	0.18	9.60E-01	0.99
					SHIP	1717	406	0.82	-0.10	0.10	3.02E-01	0.91
					Nine study meta-analysis	**25007**	**8444**	**0.83**	**-0.10**	**0.02**	**1.61E-06**	**0.90**
					Whitehall II	4006	151	0.84	-0.14	0.15	3.47E-1	0.87
					ELSA	4727	395	0.83	0.17	0.10	9.67E-2	1.19
					ROS	810	404	0.84	-0.20	0.09	3.31E-02	0.82
					MAP	888	346	0.84	-0.02	0.10	8.76E-01	0.99
					Meta-analysis of 4 replication studies	**10531**	**1296**	**0.84**	**-0.04**	**0.05**	**4.32E-01**	**0.96**
					Thirteen study meta-analysis	**35438**	**9740**	**0.83**	**-0.09**	**0.02**	**2.08E-06**	**0.92**

SNPID	Chr	position	A1	A2	Study	N	Deaths	Frequency (Allele 1)	beta	se	p-value	HR
rs12042640	1	63139384	T	C	RS	5974	3174	0.30	0.12	0.03	1.02E-05	1.13
					CHS	3267	1718	0.27	0.06	0.04	1.69E-01	1.06
					FHS	3136	654	0.27	0.08	0.07	2.25E-01	1.08
					ARIC	4511	1108	0.27	0.09	0.05	6.55E-02	1.09
					AGES	3219	558	0.30	-0.04	0.07	5.58E-01	0.96
					HABC	902	183	0.27	0.16	0.07	3.09E-02	1.17
					BLSA	620	183	0.27	-0.15	0.12	2.18E-01	0.86
					InCHIANTI	1661	460	0.27	0.20	0.12	1.03E-01	1.22
					SHIP	1717	406	0.30	-0.01	0.08	9.24E-01	0.99
					Nine study meta-analysis	**25007**	**8444**	**0.28**	**0.08**	**0.02**	**1.71E-06**	**1.09**
					Whitehall II	3981	149	0.28	-0.09	0.13	4.99E-01	0.91
					ELSA	4729	393	0.28	0.10	0.08	2.21E-01	1.10
					ROS	910	404	0.28	-0.09	0.08	2.54E-01	0.91
					MAP	888	346	0.28	-0.19	0.08	2.60E-02	0.83
					Meta-analysis of 4 replication studies	**10508**	**1292**	**0.28**	**-0.06**	**0.04**	**1.80E-01**	**0.94**
					Thirteen study meta-analysis	**35415**	**9736**	**0.28**	**0.06**	**0.02**	**7.58E-05**	**1.07**
rs3128591	9	136741940	A	G	RS	5974	3174	0.75	-0.05	0.03	6.73E-02	0.95
					CHS	3267	1718	0.76	-0.10	0.04	1.10E-02	0.90
					FHS	3136	654	0.77	-0.07	0.07	3.24E-01	0.94
					ARIC	4511	1108	0.79	-0.05	0.06	3.86E-01	0.95
					AGES	3219	558	0.70	-0.09	0.06	1.57E-01	0.91
					HABC	902	183	0.75	-0.24	0.07	8.07E-04	0.79
					BLSA	620	183	0.76	-0.16	0.12	1.75E-01	0.85
					InCHIANTI InChianti	1661	460	0.74	-0.12	0.13	3.54E-01	0.89
					SHIP	1717	406	0.78	-0.09	0.09	2.67E-01	0.91
					Nine study meta-analysis	**25007**	**8444**	**0.75**	**-0.08**	**0.02**	**3.64E-06**	**0.92**
					Whitehall II	3990	153	0.75	-0.12	0.13	3.41E-01	0.89
					ELSA	4722	396	0.75	0.09	0.09	3.08E-01	1.09
					ROS	810	404	0.78	0.13	0.09	1.65E-01	1.14
					MAP	888	346	0.78	0.07	0.10	4.60E-01	1.07
					Meta-analysis of 4 replication studies	**10510**	**1299**	**0.76**	**0.06**	**0.05**	**1.88E-01**	**1.06**
					Thirteen study meta-analysis	**35417**	**9743**	**0.76**	**-0.07**	**0.02**	**1.00E-04**	**0.97**

Table S9 Diagnostic criteria for diseases considered in analysis of time to event

Study	Diagnosis of Hip Fracture
Rotterdam	Review of medical records by two independent physicians. In case of disagreement a discussion followed and senior staff reviewed the classification according to ICD10(Schuit, et al., 2004).
CHS	Hip fracture from ICD-9 codes.
Framingham	Hip fractures are confirmed with medical records for all Framingham cohorts and adjudicated by one or more physicians when necessary& defined as a fracture of the proximal femur(Kiel, et al., 1987).
ARIC	(NA)
AGES	Cases are based on self-report and ICD-10 codes from hospital records.
HABC	Participants were contacted every 6 months at clinic visits or in telephone interviews. Vital status, functional limitations, all hospitalizations, major outpatient procedures, and new disease diagnoses were ascertained. When an event was reported, hospital records were collected and evaluated by a Health ABC Disease adjudicator at each clinical site. All reported hip fractures were confirmed by radiographic report. A central committee adjudicated all deaths for immediate and underlying causes of death through death certificates, hospital records, and a proxy interview. All participants provided informed written consent for participation. The institutional review boards of the clinical sites and the San Francisco Coordinating Center at the University of California at San Francisco approved the study protocol.
BLSA	All cases are based on physical examination by a physician (ICD9).
InCHIANTI	Based on self report and physical examination by physician.

Study	Diagnosis of Stroke
Rotterdam	Review of medical records by two independent physicians. In case of disagreement a discussion followed and senior staff reviewed the classification according to ICD10(Bos, et al., 2006).
CHS	Adjudicated by a committee (methods published).(Ives, et al., 1995)
Framingham	Stroke is adjudicated by a panel of study neurologists based on medical record review and include all stroke subtypes (ischemic, hemorrhagic, unknown). TIAs were excluded.
ARIC	Based on hospital surveillance, and then medical record review.
AGES	Cases are based on ICD-10 codes from hospital record findings, from Magnetic Resonance Images of the brain, as well as self-report of stroke or TIA.
HABC	Participants were contacted every 6 months at clinic visits or in telephone interviews. Vital status, functional limitations, all hospitalizations, major outpatient procedures, and new disease diagnoses were ascertained. When an event was reported, hospital records were collected and evaluated by a Health ABC Disease adjudicator at each clinical site. All reported hip fractures were confirmed by radiographic report. A central committee adjudicated all deaths for immediate and underlying causes of death through death certificates, hospital records, and a proxy interview. All participants provided informed written consent for participation. The institutional review boards of the clinical sites and the San Francisco Coordinating Center at the University of California at San Francisco approved the study protocol.
BLSA	All cases are based on physical examination by a physician (ICD9).
InCHIANTI	Based on self report and physical examination by physician.

cont.

Study	Diagnosis of Dementia
Rotterdam	Review of medical records by two independent physicians. In case of disagreement a discussion followed and senior staff reviewed the classification according to ICD10(Devore, et al., 2009).
CHS	Dementia is from an Ancillary study, when possible, and supplemented by other information collected on study, including a proxy report of dementia and ICD-9 codes.
Framingham	Dementia Study investigators including both a neurologist and a neuropsychologist, make the determination of dementia. Participants are screened at routine FHS exams with MMSE and participants with possible cognitive impairment are invited to undergo a battery of neuro-psychological examinations. The criteria used for a diagnosis of dementia were similar to the DSM-IV criteria, which require memory impairment, with a decline in at least one other area of significant functional impairment or cognitive functioning. In addition, these deficits needed to be present for at least 6 months before a diagnosis is made.
ARIC	(NA)
AGES	Dementia diagnosis is based on a three-step diagnosis protocol. The first stage of screening consists of Digit Symbol Substitution Test (score of 17 or lower) and the Mini-Mental State Examination (score of 23 or lower). Participants who failed the first step were screened at their next clinic visit, approximately two weeks after their first visit, using the Trail Making Test (score of 8 or more for Trails B/Trails A) and the Rey Auditory Verbal Learning Test (score of 18 or lower). Participants who failed the second step underwent a neurological examination and had an interview of a designated proxy. Diagnosis of dementia was made according to international guidelines, Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV) (28) by a geriatrician, neurologist, neuropsychologist, and neuroradiologist using all cognitive testing conducted, brain MRI, and other clinical assessmens conducted routinely in the study.
HABC	Participants were contacted every 6 months at clinic visits or in telephone interviews. Vital status, functional limitations, all hospitalizations, major outpatient procedures, and new disease diagnoses were ascertained. When an event was reported, hospital records were collected and evaluated by a Health ABC Disease adjudicator at each clinical site. All reported hip fractures were confirmed by radiographic report. A central committee adjudicated all deaths for immediate and underlying causes of death through death certificates, hospital records, and a proxy interview. All participants provided informed written consent for participation. The institutional review boards of the clinical sites and the San Francisco Coordinating Center at the University of California at San Francisco approved the study protocol.
BLSA	Diagnosis of dementia by a panel of neuropsychologist, neurologist, and radiologist.
InCHIANTI	Diagnosis available at baseline only (MMSE available at follow-up periods).

Study	Diagnosis of Myocardial Infarction
Rotterdam	Review of medical records by two independent physicians. In case of disagreement a discussion followed and senior staff reviewed the classification according to ICD10 (Bos, et al., 2006).
CHS	adjudicated by a committee (methods published).(Ives, et al., 1995)
Framingham	based on review of medical records and adjudicated by a panel of 3 senior investigators using established criteria.
ARIC	based on hospital surveillance, and then medical record review.
AGES	Cases are based on ICD-10 codes from hospital records, evidence of MI from ECG collected in the first AGES examination, or from self-report.
HABC	Participants were contacted every 6 months at clinic visits or in telephone interviews. Vital status, functional limitations, all hospitalizations, major outpatient procedures, and new disease diagnoses were ascertained. When an event was reported, hospital records were collected and evaluated by a Health ABC Disease adjudicator at each clinical site. All reported hip fractures were confirmed by radiographic report. A central committee adjudicated all deaths for immediate and underlying causes of death through death certificates, hospital records, and a proxy interview. All participants provided informed written consent for participation. The institutional review boards of the clinical sites and the San Francisco Coordinating Center at the University of California at San Francisco approved the study protocol.
BLSA	All cases are based on physical examination by a physician (ICD9) .
InCHIANTI	Based on self report and physical examination by physician.

cont.

Study	Diagnosis of Heart Failure
Rotterdam	Review of medical records by two independent physicians. In case of disagreement a discussion followed and senior staff reviewed the classification according to ICD10(Bleumink, et al., 2004).
CHS	adjudicated by a committee (methods published).(Ives, et al., 1995)
Framingham	based on review of medical records and adjudicated by a panel of 3 senior investigators using established criteria. Non-hospitalized cases of Heart Failure were excluded.
ARIC	based on hospital surveillance, and then medical record review
AGES	Cases are based on ICD-10 codes from hospital records or from self-report.
HABC	Participants were contacted every 6 months at clinic visits or in telephone interviews. Vital status, functional limitations, all hospitalizations, major outpatient procedures, and new disease diagnoses were ascertained. When an event was reported, hospital records were collected and evaluated by a Health ABC Disease adjudicator at each clinical site. All reported hip fractures were confirmed by radiographic report. A central committee adjudicated all deaths for immediate and underlying causes of death through death certificates, hospital records, and a proxy interview. All participants provided informed written consent for participation. The institutional review boards of the clinical sites and the San Francisco Coordinating Center at the University of California at San Francisco approved the study protocol.
BLSA	All cases are based on physical examination by a physician (ICD9).
InCHIANTI	Based on self report and physical examination by physician.

Study	Diagnosis of Cancer
Rotterdam	Review of medical records by two independent physicians. In case of disagreement a discussion followed and senior staff reviewed the classification according to ICD10. Linkage to Rotterdam Cancer Registry(van der Klift, et al., 2003).
CHS	Determined by ICD-9 codes.
Framingham	Nearly, all cancer cases are confirmed with pathology reports and classified using the ICD-O coding.
ARIC	Cancer cases were based on cancer registry information and additionally self-report followed by medical record review.
AGES	Cases are based on ICD-10 codes from hospital records or from self-report.
HABC	Participants were contacted every 6 months at clinic visits or in telephone interviews. Vital status, functional limitations, all hospitalizations, major outpatient procedures, and new disease diagnoses were ascertained. When an event was reported, hospital records were collected and evaluated by a Health ABC Disease adjudicator at each clinical site. All reported hip fractures were confirmed by radiographic report. A central committee adjudicated all deaths for immediate and underlying causes of death through death certificates, hospital records, and a proxy interview. All participants provided informed written consent for participation. The institutional review boards of the clinical sites and the San Francisco Coordinating Center at the University of California at San Francisco approved the study protocol.
BLSA	All cases are based on physical examination by a physician (ICD9).
InCHIANTI	Based on self report and physical examination by physician.

Table S10 Descriptive statistics of 8 cohorts participating in the analysis of time to event

Study	N	N deceased or event	Mean age at Baseline (±SD)	Sex, % female	Mean follow-up time in years (±SD)
RS	4183	2110	67.4 (8.2)	61%	10.6 (4.9)
CHS	2857	2076	72.2 (5.3)	60%	9.4 (5.2)
FHS	2381	745	68.3 (9.60)	58%	4.9 (2.1)
ARIC	3862	1210	59.3 (2.9)	49%	10.7 (3.3)
AGES	1398	248	75.2 (5.2)	68%	4.3 (1.3)
HABC	1029	412	73.5 (2.8)	53%	6.5 (2.5)
BLSA	599	281	62.1 (8.9)	41%	14.4 (8.3)
InCHIANTI**	686	232	76.6 (8.2)	58%	5.5 (1.1)
TOTAL	16995	7314	67.7 (8.5)	56%	8.8 (5.7)

**please note for InCHIANTI, the morbidity was given as yes/no at follow up period (with no date of onset of disease). So the follow up is either 3 or 6 years

Table S11 Observed events (cases) within 8 study cohorts participating in the time to event

Study	N	% Alive/no event	% Death	% Hip fracture	% Stroke	% Dementia	% Myocardial infarction	% Heart failure	% Cancer
RS	4183	49.6%	13.3%	2.2%	6.5%	7.3%	4.0%	7.8%	9.3%
CHS	2857	27.3%	10.0%	5.3%	9.0%	12.3%	10.2%	11.1%	14.7%
FHS	2381	68.7%	5.0%	1.0%	3.4%	1.4%	4.1%	3.6%	12.9%
ARIC	3862	68.3%	2.6%	NA	3.3%	NA	5.0%	4.7%	16.2%
AGES	1398	82.3%	6.7%	1.4%	1.1%	0.6%	1.6%	2.3%	3.9%
HABC	1029	59.5%	4.2%	3.7%	0.3%	NA	5.3%	12.1%	14.6%
BLSA	599	74.1%	5.0%	2.0%	3.0%	NA	6.0%	5.0%	4.0%
InCHIANTI	686	53.1%	28.0%	1.0%	0.2%	3.2%	2.2%	1.0%	11.5%
TOTAL	16995	56.2%	8.4%	2.1%	4.6%	4.3%	5.3%	6.6%	12.3%

Table S12 Extended table of association results for the analysis of time to event with $p < 1 \times 10^{-4}$

Nr.	SNP	Chr	Position	Closests Reference Gene	Distance (bp) from closest gene	Coded Allele	Non-coded Allele	Frequency coded allele	HR	P-value	Study Effect Direction	Number of Supporting SNPs
2	rs10412199	19	3878771	ATCAY	307	A	G	0.3315	0.9088	3.02E-06	--?-++--	6
3	rs16852912	3	170169370	MECOM	114610	T	C	0.0767	1.1775	3.37E-06	++++-+-+	72
4	rs8001976	13	47285723	SUCLA2	129069	T	C	0.4414	1.0896	3.43E-06	+++-++--	173
5	rs11162963	1	80507169	ELTD1	1262086	T	C	0.6284	1.0924	4.15E-06	++++++++	40
6	rs4764043	12	14006749	GRIN2B	17570	T	C	0.0779	1.1735	6.10E-06	++++++++	2
7	rs3112530	5	152619870	GRIA1	230628	A	G	0.0753	0.8479	6.79E-06	----+---	130
8	rs10202497	2	237935633	COL6A3	38233	A	C	0.1426	0.8913	8.22E-06	--------	36
9	rs2367725	1	43988415	ST3GAL3	42611	T	C	0.4156	1.0786	9.31E-06	++++++-+	119
10	rs12600908	17	70839969	GRB2	14218	A	G	0.138	1.1123	1.32E-05	++++++-+	13
11	rs2287047	2	102140486	IL1R1	3653	A	G	0.3008	1.0829	1.35E-05	+++--+++	120
12	rs10771498	12	29263896	MLSTD1	3968	T	G	0.6349	0.9234	1.35E-05	------+-	196
13	rs7599033	2	118867162	INSIG2	283096	T	C	0.1314	1.1236	1.47E-05	++++++-+	82
14	rs4870508	6	157478885	ARID1B	93208	A	T	0.8119	1.1148	1.48E-05	+++++++-	7
15	rs1426435	12	114145881	TBX3	539529	T	C	0.2438	1.092	1.54E-05	++++++-+	50
16	rs6792324	3	68179094	FAM19A1	42951	A	T	0.8199	0.9034	1.61E-05	--------	49
17	rs2909678	7	130984541	PODXL	92625	T	C	0.1154	1.1359	1.73E-05	++++++--	16
18	rs7955818	12	83274376	SLC6A15	503023	A	G	0.6343	0.9223	1.83E-05	-------+	276
19	rs12917162	15	89829161	SV2B	189509	C	G	0.8678	0.8953	1.89E-05	--------	15
20	rs5749626	22	32263970	LARGE	264908	T	C	0.4519	0.9296	2.06E-05	--------	49
21	rs10778652	12	107568852	CORO1C	3545	C	G	0.4377	1.0804	2.18E-05	++++++++	44
22	rs12571753	10	119606867	RAB11FIP2	147551	A	G	0.9691	1.4306	2.23E-05	+??+++++	5
23	rs7652939	3	61262180	FHIT	50016	A	G	0.3662	1.0946	2.24E-05	+?++-++-	8
24	rs1383582	8	21537255	GFRA2	56556	A	G	0.0416	2.2129	2.46E-05	?????+?+	0
25	rs4799712	18	29527469	ASXL3	53928	A	G	0.5578	1.0772	2.54E-05	++++++--	166
26	rs10062090	5	81905921	LOC92270	256019	A	G	0.9885	0.6907	2.85E-05	-?--?--+	0
27	rs9944366	16	6620737	A2BP1	611605	C	G	0.2323	1.0862	2.93E-05	+++++-++	36
28	rs1198572	1	98269764	DPYD	110561	A	C	0.2027	1.1035	3.14E-05	+++++++-	212
29	rs39263	7	89608280	STEAP1	13344	A	T	0.5181	0.9253	3.16E-05	------+-	263
30	rs1163900	12	17503884	FLJ22655	621185	A	G	0.2491	1.0878	3.30E-05	++++++++	44
31	rs7946324	11	96739622	JRKL	973247	A	G	0.8637	1.1113	3.33E-05	++++++-+	38

32	rs12603777	17	48315590	CA10	723214	T	G	0.7936	1.1142	3.42E-05	+?++++++	49
33	rs9287696	2	5382605	SOX11	367644	A	G	0.5668	0.93	3.55E-05	--------	27
34	rs7788972	7	19000805	HDAC9	2704	A	T	0.1963	1.0932	3.73E-05	++++++++	24
35	rs12510100	4	134047050	PCDH10	242869	A	G	0.7065	1.0829	3.89E-05	++++-+++	128
36	rs17433057	2	14953718	FAM84A	259820	T	C	0.3742	1.081	4.02E-05	++++++++	104
37	rs4731030	7	121070314	LOC392979	118570	A	G	0.3861	0.9009	4.37E-05	-?------	7
38	rs310020	16	79978576	GAN	7135	A	G	0.6332	0.9292	4.45E-05	--------	54
39	rs9486692	6	108214454	SCML4	14306	T	C	0.9182	1.1404	4.61E-05	++++++++	12
40	rs7705041	5	128887267	ADAMTS19	63266	A	T	0.0438	0.8337	4.62E-05	---+----	16
41	rs2244300	1	17730188	ARHGEF10L	8728	A	G	0.502	0.9283	4.73E-05	------++	29
42	rs3782814	12	3205386	TSPAN9	60604	A	G	0.3044	1.0771	4.75E-05	++++++++	9
43	rs7705488	5	23658964	PRDM9	94501	T	C	0.0742	1.1457	5.10E-05	++++++++	104
44	rs10964842	9	21101840	IFNW1	28790	A	C	0.0457	1.2373	5.64E-05	+?++---+	10
45	rs6480129	10	67463237	CTNNA3	113301	A	T	0.2052	1.0875	5.66E-05	++++++++	74
46	rs1563361	15	92630629	MCTP2	11869	T	C	0.4383	1.0732	6.25E-05	++++-+++	8
47	rs14576	3	16243990	GALNTL2	1163	A	C	0.3638	0.9286	6.54E-05	--+-----	18
48	rs2970848	4	23426125	PPARGC1A	23383	A	G	0.6953	0.9302	6.84E-05	-----+-+	22
49	rs12791616	11	59012630	OR4D10	10216	T	C	0.1176	0.8735	6.86E-05	-?-----+	12
50	rs1342007	6	93453227	EPHA7	554634	T	C	0.79	0.9221	6.94E-05	-----+--	20
51	rs6033014	20	10963459	JAG1	360869	T	C	0.381	1.0783	6.98E-05	+++-+--+	0
52	rs1110784	18	75238190	ATP9B	1078	A	G	0.1326	1.103	7.04E-05	++-++++-	91
53	rs7265615	20	20071624	CRNKL1	86934	T	G	0.2529	0.9212	7.06E-05	------+-	21
54	rs2744704	1	22376137	WNT4	34031	A	T	0.5628	0.9324	7.16E-05	-------+	32
55	rs481357	1	120002366	ZNF697	10453	T	C	0.3337	1.0734	7.19E-05	++++++-+	42
56	rs11613501	12	125220834	TMEM132B	511292	T	C	0.1272	1.1186	7.38E-05	++++--++	20
57	rs13029552	2	133674159	NAP5	368342	A	C	0.8982	0.8775	7.43E-05	-?+---++	8
58	rs10765518	11	91440357	FAT3	284552	C	G	0.5765	1.0822	7.50E-05	+?++-++-	159
59	rs9290488	3	175172473	NLGN1	311337	T	C	0.2735	0.9251	7.60E-05	--------	97
60	rs12791495	11	24066891	LUZP2	408240	A	G	0.4923	0.929	7.81E-05	--------	8
61	rs17096151	1	75197717	LHX8	168989	T	C	0.1302	0.8977	8.45E-05	--------	60
62	rs6545764	2	59965729	BCL11A	566076	T	C	0.0468	1.1661	8.75E-05	++++-++-	25
63	rs4632347	2	112538555	TMEM87B	9271	A	G	0.501	0.9362	8.76E-05	------+-	103
64	rs10256177	7	152585452	ACTR3B	402056	A	G	0.9264	1.1618	8.81E-05	++++++++	33
65	rs1529090	2	177643317	HNRPA3	142350	A	G	0.9113	0.8879	9.02E-05	----+-++	68
66	rs7919381	10	125692361	CPXM2	50871	T	C	0.4469	1.0916	9.03E-05	++++++-+	7

67	rs10879110	12	68886951	CNOT2	36092	A	G	0.0196	1.3347	9.28E-05	+?++++-+	16
68	rs17323370	5	164168669	MAT2B	1289766	A	G	0.9218	0.8482	9.32E-05	-?--+-++	3
69	rs200247	6	50509439	TFAP2D	279776	A	T	0.9593	0.7681	9.40E-05	-??-----	3
70	rs9898180	17	48469648	KIF2B	785589	A	G	0.874	1.1394	9.56E-05	+?++-++-	2
71	rs215939	6	85523005	TBX18	7613	A	C	0.2669	1.0767	9.84E-05	+++++++-	139

p-values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36.
Chr=chromosome, Hazard Ratios (HR) are for each additional coded allele
Number of supporting SNPs: the number of SNPs within 500kb of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 (r2>=0.10) and have association p-value<0.05.
Study Effect Direction: study-specific information is presented in the order: RS, CHS, FHS, ARIC, AGES, HABC, BLSA, InCHIANTI
Direction: + = coded allele increases risk of mortality, - = coded allele decreases risk of mortality, ?= not tested

*SNPs with a MAF < 3% are not displayed

Table S13 PANTHER Pathway Analysis (time to death)

	H. sapiens (Reference)	Nr genes observed	Nr genes expected	-/+	p-value unadjusted	p-value adjusted*
Pathway						
Unclassified	22436	658	728.72	-	1,44E-12	2,40E-10
Wnt signaling pathway	348	36	11.30	+	2,7E-09	4,48E-07
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	149	19	4.84	+	7,8E-07	1,29E-04
5HT2 type receptor mediated signaling pathway	71	13	2.31	+	9,35E-07	1,55E-04
Alzheimer disease-amyloid secretase pathway	77	13	2.50	+	2,25E-06	3,74E-04
Oxytocin receptor mediated signaling pathway	62	11	2.01	+	8,46E-06	1,40E-03
Cadherin signaling pathway	168	18	5.46	+	1,56E-05	2,59E-03
Thyrotropin-releasing hormone receptor signaling pathway	61	10	1.98	+	4,16E-05	6,90E-03
PDGF signaling pathway	189	18	6.14	+	6,96E-05	1,16E-02
Biological Process						
Biological process unclassified	11321	238	367,71	-	1,29E-20	4,00E-19
Developmental processes	2152	152	69,9	+	1,39E-19	4,32E-18
Neuronal activities	569	65	18,48	+	8,94E-18	2,77E-16
Signal transduction	3406	199	110,63	+	9,09E-17	2,82E-15
Neurogenesis	587	64	19,07	+	1,43E-16	2,84E-14
Ectoderm development	692	68	22,48	+	2,33E-15	3,38E-13
Cell adhesion	622	57	20,2	+	7.00E-12	2,17E-10
Synaptic transmission	279	35	9,06	+	3,17E-11	4,60E-09
Cell communication	1213	79	39,4	+	6,79E-09	9,84E-07
mRNA transcription regulation	1459	87	47,39	+	5,80E-08	1,15E-05
Cell surface receptor mediated signal transduction	1638	94	53,2	+	9,29E-08	1,35E-05
Mesoderm development	551	42	17,9	+	5,89E-07	8,54E-05
Neuromuscular synaptic transmission	23	8	0,75	+	1,21E-06	2,39E-04
Intracellular signaling cascade	871	56	28,29	+	1,71E-06	2,49E-04
mRNA transcription	1914	100	62,17	+	2,37E-06	3,44E-04
Cation transport	482	37	15,66	+	2,34E-06	4,64E-04
Other neuronal activity	136	16	4,42	+	1,51E-05	2,18E-03
Cell adhesion-mediated signaling	379	30	12,31	+	7,00E-12	2,33E-03
Nucleoside, nucleotide and nucleic acid metabolism	3343	147	108,58	+	8,89E-05	2,76E-03
Transport	1306	68	42,42	+	1,18E-04	3,66E-03
Ion transport	616	40	20,01	+	4,19E-05	6,07E-03
Cell proliferation and differentiation	1028	55	33,39	+	2,77E-04	8,60E-03
Other intracellular signaling cascade	225	20	7,31	+	7,17E-05	1,42E-02
Oncogenesis	472	30	15,33	+	5,17E-04	1,60E-02
Molecular Function						
Molecular function unclassified	10934	231	355,14	-	3,04E-19	8,81E-18
Cell adhesion molecule	395	35	12,83	+	1,82E-07	5,28E-06
Ion channel	357	32	11,6	+	4,75E-07	1,38E-05
Voltage-gated ion channel	145	20	4,71	+	1,20E-07	1,93E-05
Transcription factor	2052	105	66,65	+	3,21E-06	9,30E-05
Receptor	1512	81	49,11	+	9,75E-06	2,83E-04
Voltage-gated calcium channel	30	8	0,97	+	8,30E-06	1,66E-03
Homeobox transcription factor	249	23	8,09	+	1,18E-05	1,90E-03

Signaling molecule	795	47	25,82	+	8,68E-05	2,52E-03
Cell junction protein	99	12	3,22	+	1,29E-04	3,75E-03
Other signaling molecule	259	21	8,41	+	1,67E-04	2,69E-02
Other cell junction protein	36	7	1,17	+	2,11E-04	3,40E-02

*Reference includes 25431 NCBI: H. sapiens genes, as used in PANTHER 6.1

Table S14 PANTHER Pathway Analysis (time to event)

	H. sapiens (Reference)	Nr genes observed	Nr genes expected	-/+	p-value unadjusted	p-value adjusted*
Pathway						
Unclassified	22436	550	599,03	-	3,58E-08	5,94E-06
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	149	16	3,98	+	4,14E-06	6,86E-04
Wnt signaling pathway	348	26	9,29	+	4,17E-06	6,92E-04
Cadherin signaling pathway	168	17	4,49	+	4,53E-06	7,52E-04
Dopamine receptor mediated signaling pathway	84	10	2,24	+	1,13E-04	1,88E-02
Integrin signalling pathway	227	17	6,06	+	1,80E-04	2,99E-02
Biological Process						
Developmental processes	2152	115	57,46	+	1,02E-12	3,16E-11
Biological process unclassified	11321	214	302,27	-	2,93E-12	9,08E-11
Neuronal activities	569	47	15,19	+	2,28E-11	7,08E-10
Neurogenesis	587	48	15,67	+	1,95E-11	3,86E-09
Signal transduction	3406	151	90,94	+	2,22E-10	6,87E-09
Ectoderm development	692	51	18,48	+	1,67E-10	2,42E-08
mRNA transcription regulation	1459	82	38,95	+	2,90E-10	5,74E-08
Cell communication	1213	71	32,39	+	9,76E-10	1,42E-07
mRNA transcription	1914	92	51,1	+	4,33E-08	6,28E-06
Cell adhesion	622	41	16,61	+	7,41E-07	6,41E-06
Cell adhesion-mediated signaling	379	29	10,12	+	2,07E-07	1,47E-04
Cell surface receptor mediated signal transduction	1638	77	43,73	+	1,41E-06	2,05E-04
Synaptic transmission	279	23	7,45	+	3,11E-06	4,52E-04
Nucleoside, nucleotide and nucleic acid metabolism	3343	128	89,26	+	1,79E-05	5,53E-04
Cell proliferation and differentiation	1028	51	27,45	+	2,41E-05	7,48E-04
Cell structure and motility	1148	53	30,65	+	1,05E-04	3,26E-03
Apoptosis	531	27	14,18	+	1,38E-03	4,27E-02
Molecular Function						
Molecular function unclassified	10934	195	291,93	-	1,13E-14	3,27E-13
Transcription factor	2052	91	54,79	+	1,62E-06	4,71E-05
Cell adhesion molecule	395	28	10,55	+	4,76E-06	1,38E-04
Receptor	1512	67	40,37	+	4,41E-05	1,28E-03
Nucleic acid binding	2850	110	76,09	+	5,69E-05	1,65E-03
Signaling molecule	795	40	21,23	+	1,36E-04	3,95E-03
Other cell junction protein	36	7	0,96	+	6,38E-05	1,03E-02
CAM family adhesion molecule	72	9	1,92	+	1,72E-04	2,77E-02
Other transcription factor	349	22	9,32	+	2,48E-04	4,00E-02
Cadherin	111	11	2,96	+	2,53E-04	4,07E-02

*Reference includes 25431 NCBI: H. sapiens genes, as used in PANTHER 6.1

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3. Disability, Mortality, and Life Expectancy

CHAPTER 3.1

Physical Activity and Disability: Intensity of Activity Matters

Stefan Walter, Henning Tiemeier, Albert Hofman, Johan Mackenbach

CHAPTER 3.2

Mortality and disability - the impact of overweight and obesity

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International Journal of Obesity. 2009; **33,** 1410–1418

ABSTRACT

Context: Prevalence of obesity is increasing globally. The impact of obesity on mortality and morbidity and its implication on the future prevalence of disability in the elderly population has not been conclusively investigated.

Objective: To determine the influence of overweight and obesity on mortality and disability by quantifying the effect in terms of disability free life expectancy and years lost to disability in the elderly.

Design, Setting, and Participants: For 5980 participants from the Rotterdam Study Cohort, regression techniques were used to estimate the association of body mass index and waist circumference separately with mortality, incident disability, and recovery from disability. Disability was assessed using the Stanford Health Assessment Questionnaire Disability Index (HAQ-DI), an Activity of Daily Living (ADL) scale. Multi-state life table methodology was used to calculate life expectancies.

Main Outcome Measures: 15 year mortality risk, 6 year disability incidence, total life expectancy, healthy life expectancy, and years of disabled life expectancy.

Results: We observed 2388 deaths. Our analysis revealed no association between body mass index, or waist circumference and mortality in the healthy population. Body mass index and waist circumference were related to disability ('overweight' $25 \leq BMI < 30$, OR=1.33, 95%-CI [1.10; 1.61], 'obesity I' $30 \leq BMI < 35$, OR=2.03, 95%-CI [1.55; 2.65]) and negatively to recovery from disability. We observed an increase of years lost to disability with increasing weight for men ('normal weight' – 4.69y, 'overweight' – 5.87y, 'obesity I' – 7.06y) and for women ('normal weight' – 10.95y, 'overweight' – 12.82y, 'obesity I' – 15.17y, 'obesity II/III' – 13.13y).

Conclusion: Results do not support the hypothesis that an increased body weight reduces total life expectancy in the elderly. Although increased body weight was associated with a higher risk of becoming and remaining disabled. These results remained using waist circumference.

Introduction

Overweight and obesity are important determinants of mortality and disability. The increasing prevalence of obesity contributes to a reduction in quality of life. In Europe, more than half of all adults are overweight.(1) Obesity prevalence in the Netherlands has increased dramatically in the last decades and effective strategies to alleviate the societal burden of obesity are needed.(2),(3)

While the literature agrees on the inverse association of obesity and overweight on mortality in young adulthood and middle age,(4),(5),(6),(7) consensus is limited when focusing on the elderly population.(8),(9),(10),(11) Research showed an association between increased body mass index (BMI) and the incidence and prevalence of disability .(12),(13)

Depending on the balance between mortality and disability risks due to increased body weight, obesity could result in compression or expansion of disability (i.e. more or less time spent with disability). Compression of disability towards the end of life is believed to reduce overall costs to society of an aging population.(14) Research that evaluated obesity in relation to both mortality and disability risks by means of multi-state life tables yielded contradicting results. The estimates of years lost to disability (YLD) due to overweight and obesity vary between 6 – 10 years depending on age at baseline and sex.(9, 11-12, 15-17) No difference in YLD attributed to increased mortality risks among obese participants in the adult population in one study(15) is contrasted by investigations that showed increased total and disability free life expectancy among obese participants as compared to the normal weight population in other studies.(9, 11-12, 16-17) Some authors argue that there is a recent change in the relationship between excess body weight, disability and mortality because of the improvements in treating cardiovascular conditions. This suggests that presently the disabling effects of obesity outweigh the mortality effects resulting in expansion of morbidity.(18),(7)

Most of the studies mentioned above used BMI as an indicator for overweight and obesity. Particularly in the elderly population waist circumference (WC) might be a better measure when evaluating obesity-related health risks and all-cause mortality.(19),(20),(21)

The aim of this study is to estimate the risk of overweight and obesity on mortality and disability and to quantify the effect in terms of disability free life expectancy (DFLE) and years lost to disability (YLD) in the elderly. Using n=5980 participants of 55 years and older from the Rotterdam Study cohort we investigated the state-specific mortality risk, disability

incidence, as well as recovery from disability as a function of BMI and WC adjusting for major confounders. Subsequently, we estimated transition probabilities using a Markov modelling approach and derived DFLE and YLD using a multi-state life table. Among the strengths of this investigation is the clinical assessment of outcomes and covariates and the long mortality follow up.

Data and Methods

The Rotterdam Study is a population-based longitudinal study designed to investigate diseases and risk factors for diseases among the elderly population of 55 years or over in the Ommoord district of Rotterdam, The Netherlands. From June 1990 to August 1993 trained research assistants collected data on health, medication use, medical and family history, and life style factors for n=7983 participants (78% of 10 215 invited to participate) in extensive home interviews. Participants subsequently visited the research center for a clinical examination. Detailed information on the design of the Rotterdam Study has been published elsewhere.(22)

The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of the Netherlands Ministry of Health, Welfare and Sports. All participants provided written informed consent.

Data analyzed in this study comprised n=5980 participants from the Rotterdam Study. We excluded participants that did not visit the research center and had no anthropometric measurements (n=1073), that had a BMI < 18:5 (n=78), or had not answered the questions regarding smoking behavior (n=162). In addition, we excluded participants who unintentionally lost more than 3.5kg in the 18 month prior to baseline measurements (n=690) to account for disease-related weight loss prior to death.

Anthropometric measures rely on clinical measurements from the baseline investigation in our research center. Height and weight were measured in participants without shoes and heavy clothing and BMI was calculated as kg/m^2. We modelled BMI as categorical variable with four categories. 'Normal weight' ranging from $18:5 \leq BMI < 25$, 'overweight' $25 \leq BMI < 30$, 'obesity I' $30 \leq BMI < 35$, and 'obesity II/III' $35 \leq BMI$ according to WHO cut-off criteria.(23) WC was measured midway between the lower rib margin and the iliac crest with the participant breathing out gently. We classified WC using different cut offs for males and

females. We constructed three classes for males (WC - <94cm, WC < 102cm, WC ≥ 102cm) and females (WC < 80cm, WC < 88cm, WC ≥ 88cm) using clinical cut-points.(24)

We analyzed the influence of BMI and WC on two outcome variables, 15 – year mortality and 6 - year disability incidence. All participants of the Rotterdam Study were under continuous surveillance; general practitioners' and hospital records as well as death certificates were used for follow-up of deceased participants till the 1st of January 2006. Disability status was assessed at baseline and follow-up center visit after six years by the Activities of Daily Living from the Stanford Health Assessment Questionnaire Disability Index (HAQ-DI).(25)

The HAQ-DI has proven to be a valid, effective, and sensitive tool for the assessment of health status.(26) The HAQ-DI consists of 8 categories: *dressing, arising, eating, hygiene, walking, reach, grip, and outside activity*. Each of these categories consists of two to four questions inquiring about the ability to perform a task `Are you able to. . . '. The status of the participant was evaluated as able to perform without difficulty (0), with some difficulty (1), with much difficulty (2) or unable to perform/requiring assistance (3). The highest scoring item determined the overall category score. The mean score of all categories was equivalent to the HAQ-DI ranging from 0.00 to 3.00. In accordance with the literature we used a HAQ-DI ≥ 0.5 to define a participant as at least mildly disabled.(13)

We used Cox proportional hazard regression to estimate the influence of BMI and WC on mortality. The associations between BMI, WC and incidence of disability and recovery from disability six years after baseline were estimated using logistic regression. The analysis revealed a significant interaction between BMI and sex ($p < 0.001$), as well as WC and sex ($p \sim 0.03$) in the logistic regression analysis of recovery from disability. We therefore decided to stratify the life table estimations by sex.

The overall impact of overweight and obesity on a persons life in terms of YLD and DFLE can only be assessed taking into consideration the risk of disability and the chances of recovery. We modelled a three-state illness-death model using the Broyden–Fletcher–Goldfarb–Shanno (BFGS) variable metric algorithm in the R msm package to account for competing risks between disability and mortality.(27),(28)

In order to increase comparability we used the following covariates in all regression models. Education was grouped into four categories 'elementary education', 'lower secondary education', 'higher secondary education', and 'tertiary education'. We assessed living situation

as a dichotomous variable describing whether the participant lived alone or not. We adjusted for income as the equivalent household income in 1000 € per month. Missing values were accounted for by defining a binary variable describing whether or not information on income was available. If the information was not available the continuous income variable was set to '0' otherwise the income was included. This method of treating missing data is adequate when controlling for limited confounding and if interpretation of the risk estimates of the confounders is no objective of the study.(29-30) A similar approach was taken for the continuous measure of alcohol consumption. Drinking behavior was similarly modelled as 'unknown', 'yes', 'no' and supplemented with information on the daily intake of alcohol in gram. Smoking status was accounted for through the states 'current smoker', 'former smoker', and 'never smoker' accompanied by a continuous measure of smoked pack years of cigarettes.

We constructed a hypothetical Rotterdam Study participant attributing the mean and modal values of the covariates used previously in the regression models to this participant. The transition probabilities from the disability-free (healthy) to the disabled state or death state and from the disabled state to disability-free or death were derived from this model and used as input for the multi-state life table. Empirical 95% confidence intervals (CIs) for the life table measures were calculated using a 250 replicate bootstrap procedure. Unless otherwise indicated all analysis are based on SAS V9.1.3 by the SAS Institute Inc.,Cary, NC, USA.

Results

Table 1 shows the baseline characteristics of the overall population as well as stratified by baseline disability status as measured by Activities of Daily Living (ADL). 4620 participants were classified as "healthy", i.e. non-disabled, and 1360 as disabled at baseline. In total, we observed 2388 deaths in our study population over 15 years of follow-up. Out of these, 1530 deaths were registered among the non-disabled population and 858 among the disabled population. People disabled at baseline were older, exhibited higher measures of anthropometry, and had a lower socio economic status as measured by education and income.

Table 2 displays the results of the Cox-regression analysis of body weight, as measured by BMI or WC, and mortality stratified by baseline ADL-disability status. In the population healthy at baseline, the hazard ratios across the categories of BMI or WC lacked statistical significance. The hazard ratio for the overweight population as classified by a BMI between 25 and 30 indicates slightly and non-significantly reduced hazards in comparison to the normal weight population (HR 0.96, 95%-CI [0.86; 1.07]). Among disabled participants,

overweight (HR 0.82, 95%-CI [0.70; 0.96]) and obesity I (HR 0.73, 95%-CI [0.60; 0.90]) were negatively associated with mortality indicating protective effects. Among the severely obese participants the hazard ratio suggested increased mortality albeit lacking statistical significance. No statistically significant effect on mortality could be observed using waist circumference as underlying measure to classify participants.

Table 3 shows the results of the logistic regression analysis for six year disability incidence and recovery from disability. For a healthy obese person the odds of being disabled six years later were roughly twice as high as compared to a person of normal weight (OR=2.03, 95%-CI [1.55 ; 2.65]). The analysis similarly indicated negative associations for BMI and recovery. Being 'overweight' reduced the odds of recovery by one third (OR=0.66, 95%-CI [0.41; 1.07]). Obesity cut the odds of recovery by more than half (OR=0.42, 95%-CI [0.22; 0.80]). When we applied WC instead of BMI a similar association with disability incidence was observed. Results for the association between WC and recovery from disability were statistically significant only in the highest WC group indicating a strong reduction of the odds of recovery for overweight and obese participants (OR=0.47, 95%-CI [0.27; 0.83]). Modelling the obesity - mortality or obesity - disability relationship using waist circumference was more appropriate than applying the BMI categories according to the Akaike Information Criterion (AIC).

The results from the life table analysis were based on the 'normal' Rotterdam study participant and refer to a healthy, i.e. non-disabled, 55 year old population at baseline. Figure 1 illustrates the joint analysis of BMI on mortality and ADL disability stratified by sex. Overall life expectancy remained largely constant except in the obesity II/III category. Disability free life expectancy decreased with increasing BMI . At the same time years lost to disability increased gradually over the BMI categories reaching its peak in the 'obesity I' category. Due to the small sample size in the obesity II/III category among male participants, reliable estimates could not be obtained. Figure 2 shows the same analysis for WC. The influence of overweight and obesity when measured by WC on total life expectancy was negligible. Disability free life expectancy decreased with increasing WC while years lost to disability increased. Table 4 shows the life table estimations numerically.

Discussion

The general objective of this study was to quantify the effect of overweight and obesity on disability free life expectancy and years lost to disability. These analyses showed a limited

impact of overweight and obesity on mortality but a significant impact on morbidity as described by incident and persistent ADL disability. As the disabling effects of overweight and obesity exceeded the mortality effects, years lost to disability increased among overweight and obese participants compared to normal weight participants. This finding was valid independent of the approach used to classify overweight and obese individuals.

Strength and Limitations

Before we discuss our findings, several limitations must be pointed out. 776 participants refused to participate in the follow up interview. Our non-response analyses showed that these persons had on average a higher BMI. We could not assess their functional status, but if the non-response was related to disability, which is not unlikely, this selection effect could have led to an underestimation of the true effect of obesity and overweight on disability in our analyses. While the estimation using MSM modelling takes into account arbitrary observation times and unknown states directly before a known transition, e.g. death, these participants did not contribute to the estimation of the transition probabilities to the disabled or non-disabled state.

We modelled disability incidence after six years. It is likely that we missed incident disability cases and recoveries within this time window. Using the Markov modelling approach we partially accounted for these missed events.(27) Sample size was another limitation especially when we estimated the hazard ratios for recovery from disability. It would have been interesting to distinguish participants in the obesity II/III category (BMI $\geq$ 35). However, severely obese participants were rare at baseline.

Further we lacked data on physical activity. The bias resulting from this should be negligible for the mortality analysis as overweight and obesity were equally predictive of mortality across different levels of physical activity level in a previous study.(31). Physical activity is associated with disability independent of overweight or obesity (32).

Common shortcomings in this type of research are mostly related to improper consideration of confounders such as smoking, sudden, disease induced weight loss, and comorbidities.(33),(34) We carefully adjusted for smoking by using smoking status and the pack years of cigarettes smoked till assessment at baseline and similarly accounted for alcohol consumption, education, and socioeconomic status in our analysis. We also excluded n=690 participants that reported unintentional weight loss of more than 3.5kg in the 18 month prior to baseline measurements to forestall the effects of disease induced weight loss on our

analysis. We did not control for disease prevalence because diseases are intermediaries in and not confounders to the association between obesity and disability and mortality

Among the strengths of our study is the assessment of the determinant. Our measurements do not rely on self reported information but on clinical measurements of BMI and WC in our research center.

Mortality

Our analysis of the association between BMI and mortality in the elderly corroborated findings already presented by others in the literature. The negative effects of overweight and obesity on survival are well documented among the young, and working age population.(35) Among the elderly population an increase in BMI is not consistently correlated with an increase in mortality risk. (8-10, 18) Overweight and obesity have been attributed with protective effects even in the healthy, elderly population(11) but another study concluded that increased BMI had a hazardous impact on survival. However, participants suffering from chronic disease exhibited a weaker, maybe even protective association between increased BMI and mortality.(36) Our analysis did not reveal protective effects of an increased BMI or increased WC in the healthy population. People disabled at baseline though seemed to benefit from overweight and even obesity when considering survival.

Why is excess body weight in the elderly not detrimental and in the disabled even protective? One hypothesis is related to medical progress. Optimized medical care for chronic diseases and in the treatment of cardiovascular conditions might have halted the negative health impact of increased levels of body fat.(7, 18) This could explain why overweight and obesity I showed no negative effects on survival our analyses, but it cannot serve as an explanation as to why we observed a positive effect on overall survival in the disabled population. It has been argued that overweight and obesity serve as a general nutritional reserve in times of stress and disease.(37-38) In addition several studies showed that increased BMI is positively associated with survival following critical care(39-40), but others did not find this association(41), or even attribute higher mortality to an increased BMI.(42)

The relationship between waist circumference and mortality is often described a J-shaped.(43-45) Increased waist circumference was found to be an independent risk factor for all cause mortality suggesting that fat distribution might be equally or more important than total body fat.(43) In one study, the authors did not report on a higher likelihood of mortality due to increased waist circumference and increased mortality (46) or only found this association in

subgroups.(19, 37) We could not confirm a higher risk of mortality with increased waist circumference in our study after stratifying for disability status. This might be due to the categorization of waist circumference in other studies. Most of the literature attributing an additional mortality risk to increased waist circumference categorized waist circumference according to study specific cut-offs. We decided to adhere to the clinical cut-offs that are included in guidelines on weight management.(24, 47)

Disability

Our analysis indicated that overweight and obesity, whether assessed by BMI or WC, increased the odds of disability. As a consequence, more time was spent with disability throughout life. While it is beyond the scope of this paper to show the impact of obesity on disease specific outcomes, it is important to realize that different diseases contribute to disability as measured by ADL.(48) Obesity is related to several musculoskeletal conditions that favour disability.(49) Among these conditions are osteoarthritis, low back pain, diffuse idiopathic skeletal hyperostosis, gait disturbance, soft tissue conditions, osteoporosis, gout, fibromyalgia, and connective tissue disorders.

Some authors investigated the association between BMI and upper- and lower limb disability.(12) They concluded that disability risk was higher for obese persons but not consistently so for overweight individuals. Our results differed and indicated a significant increase in disability risk with increasing BMI and WC. Additionally we observed multiplicative interaction between BMI, WC, and sex when analyzing recovery from disability. ADL disability is more common among women than men.(50) This is probably due to increased survival and disadvantages in recovery from disability as compared to men.(50-51). Sex differences in mortality, morbidity, and physical function have been the focus of scientific investigations. Men are physically stronger than women even at advanced ages and thus more likely to recover from mild disability.(52) The finding that overweight and obesity have a more pronounced effect among the recovery from disability among females could indicate that excess body weight paired with less muscle mass is the most hazardous phenotype.

Life Table Estimation

In our analysis overweight was associated with early disability but not with early death and there were indications that severe obesity was strongly associated with both mortality and

disability. Previous research showed that obesity was associated with a higher proportion of life spent under ADL disability in adults 70 years and older.(16) Al Snih et al. noted that 'overweight' was associated with the highest disability-free life expectancy in elderly persons.(9). We found a consistent increase in disabled life years in the overweight and obese category and could no confirm the 'overweight' category to have the highest life expectancy free of ADL disability.

A prior study suggested that declining mortality effects of obesity due to cardiovascular improvements were not accompanied by a reduction in ADL disability among the obese population.(18) Our results similarly indicated that total life expectancy at age 55 for the average Rotterdam Study participant whether male or female remained largely unaffected by overweight and obesity while the years lost to disability increased. An exception to this was found only among severely obese female participants with a BMI larger than or equal to 35.

It is an intriguing finding that while there is an average difference of five years in total life expectancy between men and women, the same difference cannot be observed when focusing on disease free life expectancy. Together with the observation of a sex-interaction with BMI and WC in the analysis of recovery from disability there is an indication that women are more prone to become (at least) mildly disabled state and that excess bodyweight might adversely affect recovery and increase the duration of disability among females more than among males.

Conclusion

Our analysis consistently showed that the risk of an increased body weight cannot be found in the domain of overall survival but rather in the onset and duration of disability. Using WC to approximate overweight and obesity might be preferable to BMI but it did not change our conclusions. Overweight and obesity were associated with early, and extended periods of disability. In addition, women might be disproportionally affected by the disability risk induced by excess body weight. If the obesity epidemic continues, we could observe an increased burden of disability among the elderly in the future. This underlines the importance of preventive actions to halt the obesity epidemic, including among the baby boom cohorts.

Author Contribution: Stefan Walter had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Walter, Kunst, Tiemeier.
Acquisition of the data: The Rotterdam Study Investigators.
Analysis and interpretation of the data: Walter, Kunst, Mackenbach, Hofman, Tiemeier.
Critical revision of the manuscript for important intellectual content: Walter, Kunst, Mackenbach, Hofman,
Tiemeier.
Statistical Analysis: Walter.
Obtainend Funding: Mackenbach, Hofman.
Study supervision: Kunst, Tiemeier.
Financial Disclosure: None reported.
Funding/Support: The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organisation of Scientific Research (NWO), the Netherlands Organisation for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, and the European Commission (DG XII). Stefan Walter is funded by NETSPAR and the NETSPAR theme "Living Longer in Good Health".
Role of the Sponsor: The sponsors had no role in the design or conduct of the study; in the collection, management, analysis, or interpretation of the data; or in the preparation, review, or approval of the manuscript.
Additional Contributions: Wilma Nusselder, PhD (Department of Public Health, Erasmus MC), Caspar Looman (Department of Public Health, Erasmus MC), and Istvan Majer (Department of Public Health, Erasmus MC) provided technical assistance in the statistical analysis.

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Figure 1 Adjusted life expectancies for average Rotterdam Study participant by BMI category

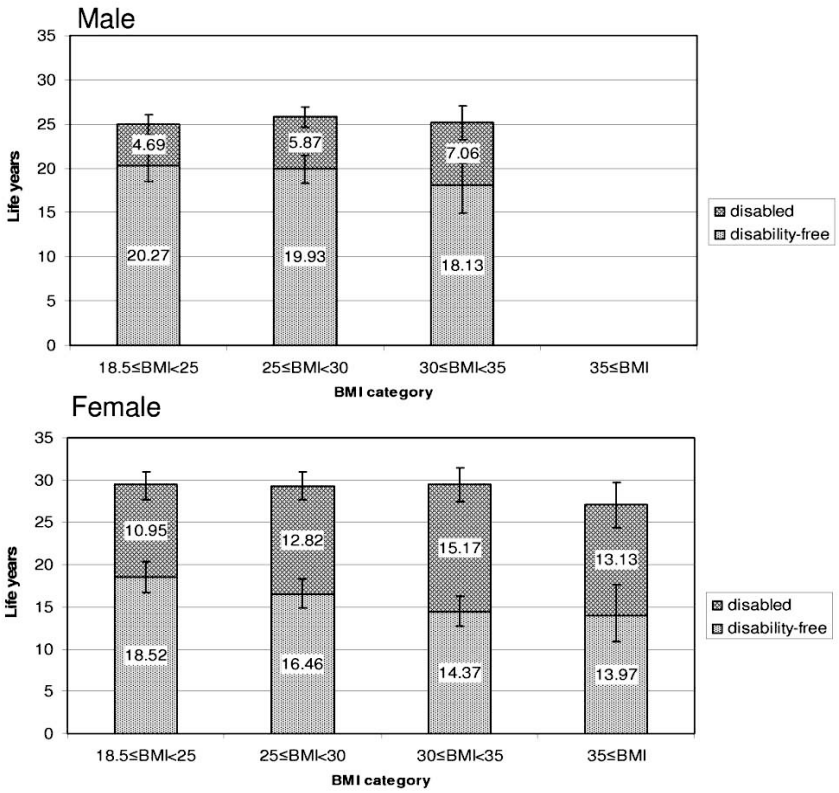

Figure 1 Life expectancies at age 55 by BMI category for the average and healthy Rotterdam Study participant stratified by sex.

Adjusted life expectancies for average Rotterdam Study participant by waist circumference category

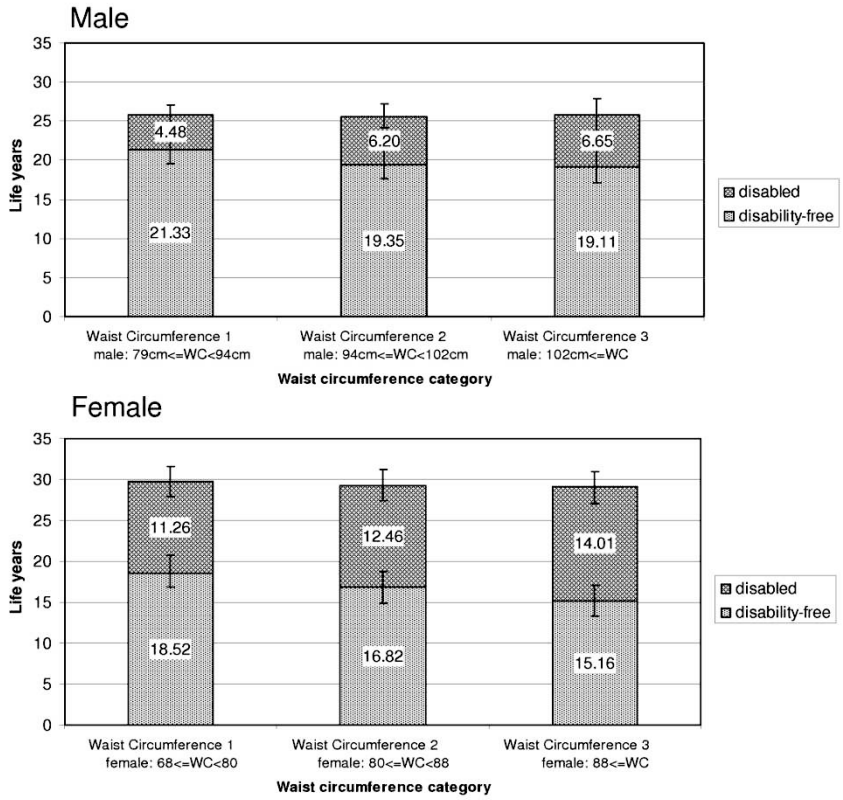

Figure 2 Life expectancies at age 55 by waist circumference category for the average and healthy Rotterdam Study participant stratified by sex.

Table 1 Baseline characteristics of study population

Label	Entire Population Mean (SD) /Frequency	Non-disabled (ADL) Mean (SD) /Frequency	Disabled (ADL) Mean (SD) /Frequency
Population			
n	5980	4620	1360
Female sex	58.63%	54.03%	74.26%
Age at interview (years)	68.85 (8.63)	66.76 (7.40)	75.07 (9.42)
Anthropometry			
Body Mass Index (kg/m2)	26.55 (3.63)	26.29 (3.41)	27.41 (4.17)
Normal (BMI 18.5 - 24.9)	34.88%	36.43%	29.63%
Overweight (BMI 25.0 - 29.9)	48.90%	49.85%	45.66%
Obesity I (BMI 30.0 -34.9)	13.80%	12.01%	19.85%
Obesity II (BMI 35.0+)	2.42%	1.71%	4.85%
Waist Circumference (cm)	90.94 (10.91)	90.15 (10.65)	93.72 (11.40)
Waist Circumference 1 male: 79cm<=WC<94cm, female: 68<=WC<80	30.92%	34.73%	17.61%
Waist Circumference 2 male: 94cm<=WC<102cm, female: 0<=WC<88	30.50%	31.97%	25.35%
Waist Circumference 3 male: 102cm<=WC, female: 88<=WC	38.59%	33.29%	57.04%
Social Economic Status			
Living Situation			
alone	27.81%	22.86%	44.63%
partner	64.92%	70.15%	47.13%
other/unknown	7.27%	6.99%	8.24%
Education			
Elementary	37.34%	32.34%	54.34%
Lower secondary	27.27%	28.98%	21.47%
Higher secondary	26.67%	28.94%	18.97%
Tertiary	8.71%	9.74%	5.22%
Income			
Income not known	14.00%	11.06%	23.97%
Income known	86.00%	88.94%	76.03%
Household equivalent income (1000 Euro / month)	2.19 (1.01)	2.27 (1.03)	1.91 (0.88)
Lifestyle Variables			
Smoking			
Smoking current	22.72%	23.66%	17.57%
Smoking former	42.71%	44.89%	35.29%
Smoking never	35.02%	31.45%	47.13%
Packyears of cigarettes smoked (20 cigarettes = pack per day)	16.19 (22.82)	17.06 (22.76)	13.25 (22.79)
Alcohol			
Alcohol unknown	18.70%	13.87%	35.07%
Alcohol no	16.34%	15.30%	19.85%
Alcohol yes	64.97%	70.82%	45.07%
Daily alcohol consumption g/day	10.52 (15.27)	11.10 (15.58)	7.89 (13.49)

Table 2 Adjusted hazard ratios for the 15-year mortality stratified by disability status*

Body Mass Index

Effect	15y - mortality - nondisabled			15y - mortality - disabled		
	Hazard Ratio	95% Confidence Limits		Hazard Ratio	95% Confidence Limits	
Age	1.118	1.11	1.127	1.14	1.101	1.126
Sex (female vs. male)	0.559	0.488	0.64	0.599	0.487	0.736
normalweight BMI [18.5;25[	1.000			1.000		
overweight BMI [25;30[vs [18.5-25[	0.962	0.862	1.074	0.818	0.698	0.958
obesity I BMI [30;35[vs [18.5-25[	1.056	0.89	1.252	0.734	0.601	0.897
obesity II/III BMI [35+[vs [18.5-25[	1.309	0.86	1.994	1.115	0.795	1.565
AIC	23645.19			21096.51		
SBC	23741.18			21185.40		

Waist Circumference

Effect	15y - mortality - nondisabled			15y - mortality - disabled		
	Hazard Ratio	95% Confidence Limits		Hazard Ratio	95% Confidence Limits	
Age	1.118	1.109	1.126	1.113	1.1	1.127
Sex (female vs. male)	0.570	0.493	0.66	0.584	0.465	0.732
Waist Circumference 1 male: 79<=WC<94, female: 68<=WC<=80	1.000			1.000		
Waist Circumference 2 male: 94<=WC<102, female: 80<=WC<88	1.052	0.923	1.199	1.014	0.803	1.281
Waist Circumference 3 male:102<=WC, female: 88<=WC	1.048	0.914	1.202	1.052	0.846	1.307
AIC	10813.73			9335.02		
SBC	10899.31			9413.56		

* All analyses were additionally adjusted for smoking status, packyears of cigarettes smoked in the past, alcohol consumption, alcohol consumption in g/day, education, income, and living situation. AIC – Akaike Information Criterion, SBC – Schwarz Bayesian Criteria

Table 3 Adjusted odds ratios for the 6y-incidence of disability*

Body Mass Index

Effect	6y- Disability Incidence			6y-Recovery from Disability		
	Odds Ratio	95% Confidence Limits		Odds Ratio	95% Confidence Limits	
Age	1.122	1.106	1.138	0.908	0.879	0.938
Sex (female vs. male)	2.095	1.672	2.625	0.548	0.310	0.968
normalweight BMI [18.5;25[	1.000			1.000		
overweight BMI [25;30[vs [18.5-25[	1.329	1.095	1.613	0.660	0.406	1.074
obesity I BMI [30;35[vs [18.5-25[	2.026	1.550	2.648	0.416	0.217	0.797
obesity II/III BMI [35 + vs [18.5-25[	2.143	1.156	3.972	0.324	0.088	1.201
AIC	3332.26			682.20		
SBC	3448.86			686.69		

Waist Circumference

Effect	6y- Disability Incidence			6y-Recovery from Disability		
	Odds Ratio	95% Confidence Limits		Odds Ratio	95% Confidence Limits	
Age	1.119	1.109	1.135	0.906	0.875	0.938
Sex (female vs. male)	2.019	1.590	2.566	0.636	0.348	1.160
Waist Circumference 1 male: 79<=WC<94, female: 68<=WC<=80	1.000			1.000		
Waist Circumference 2 male: 94<=WC<102, female: 80<=WC<88	1.477	1.175	1.857	0.897	0.500	1.609
Waist Circumference 3 male:102<=WC, female: 88<=WC	1.814	1.446	2.277	0.472	0.270	0.828
AIC	3086.75			565.03		
SBC	3195.73			644.09		

* All analyses were additionally adjusted for smoking status, packyears of cigarettes smoked in the past, alcohol consumption, alcohol consumption in g/day, education, income, and living situation. AIC – Akaike Information Criterion, SBC – Schwarz Bayesian Criteria

Table 4: Life table estimates for a normal 55 year old Rotterdam Study participant stratified by sex

	MALES						FEMALES					
Determinant	**TLE**	**95% CI**	**DFLE**	**95% CI**	**YLD**	**95% CI**	**TLE**	**95% CI**	**DFLE**	**95% CI**	**YLD**	**95% CI**
Body Mass Index												
Normalweight BMI [18.5;25[	24.96	23.41 – 26.37	20.27	18.58 - 22.00	4.69	3.54 - 5.83	29.47	28.16 - 30.76	18.52	16.75 - 20.35	10.95	9.20 - 12.50
overweight BMI [25;30[vs [18.5- 25[	25.80	24.39 – 27.20	19.93	18.41 - 21.51	5.87	4.75 - 7.05	29.28	28.08 - 30.52	16.46	14.89 - 18.30	12.82	11.22 - 14.50
obesity I BMI [30;35[vs [18.5-25[	25.19	22.87 – 27.60	18.13	15.65 - 21.34	7.06	5.19 - 8.99	29.54	28.04 - 31.11	14.37	12.67 - 16.28	15.17	13.14 - 17.07
obesity II/III BMI [35 + vs [18.5-25[	not applicable		not applicable		not applicable		27.10	24.85 - 29.80	13.97	10.84 - 17.58	13.13	10.34 - 15.77
Waist Circumference												
Waist Circumference 1 male: 79<=WC<94, female: 68<=WC<=80	25.81	24.09 - 27.73	21.33	19.55 - 23.16	4.48	3.17 - 5.79	29.78	28.18 - 31.44	18.52	16.78 - 20.68	11.26	9.40 - 13.12
Waist Circumference 2 male: 94<=WC<102, female: 80<=WC<88	25.56	23.81 - 27.17	19.35	17.61 - 21.54	6.20	4.78 - 7.81	29.29	27.62 - 30.86	16.82	14.86 - 18.79	12.46	10.62 - 14.40
Waist Circumference 3 male:102<=WC, female: 88<=WC	25.76	23.53 - 27.76	19.11	17.16 - 21.30	6.65	4.60 - 8.68	29.17	27.59 - 30.56	15.16	13.35 - 17.07	14.01	11.87 - 15.80

TLE=Total life expectancy, DFLE=Disability free life expectancy, YLD= Years lost to disability. A “normal” participant was defined as a person with mean and modal values of covariates.
*TLE, DFLE, YLD could not be estimated for obesity II/III among male participants due to limited sample size in this category.

CHAPTER 3.3

Obesity, incident disease, and mortality – the obesity paradox revisited

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CHAPTER 3.4

Is positive affect associated with Survival? A population-based study of elderly persons

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American. Journal of Epidemiology. 2011; 173 (11): 1298-1307.

Abstract

Study results on the association of positive affect with survival are conflicting. This disagreement potentially arises from poor control for health or negative affect, and the varying age groups studied. We examined if positive affect predicts survival, whether this association is preserved after controlling for negative affect, socioeconomic status, lifestyle and health, and whether this association varies with age. The study is set within the population-based Rotterdam Study and included 4411 participants aged 61 years and older, followed for on average 7.19 (SD=2.20) years.

Positive affect was not consistently associated with survival across all ages. We found a significant interaction of positive affect with age on survival (*p*-value =0.02). Subsequent age stratification revealed that positive affect independently predicted survival in elderly persons aged <80 years (HR per affect score, 0.96, 95%-CI:0.93,0.99), but not in those aged >80 years in fully adjusted models (HR 1.00; 95%-CI:0.96,1.04). In the oldest-old, the association was partly explained by differences in baseline health. In conclusion, the results suggest that there may be an association of positive affect with survival in the younger and middle-old, but not in the oldest-old in whom perception of positive affect is more likely to be determined by health.

Keywords: affect, health, ageing, survival

Introduction

Positive affect is a mental health state that can be defined as pleasurable engagement with the environment and is characterized by happiness, joy, excitement, enthusiasm or contentment.(1) Positive affect is proposed to predict good health and has been associated with several determinants of survival such as indicators of immune function. Studies showed that positive affect was related to a lower risk of the common cold (2) and less disability among patients with arthritis.(3) Also, positive affect reduced the risk of cardiovascular events such as stroke (4-5) and myocardial infarction.(5-7)

The association between positive affect and survival has previously been studied and reviewed.(1, 8-9) The majority of studies found that positive affect was associated with lower mortality rates.(10-15) However, some studies failed to find an association between positive affect and survival.(16-18) These inconsistencies may arise from the different ways to account for indicators of baseline health status and other potential confounders between these studies. Several of these studies did not control for health status or relied solely on self-reported proxies for health.(12) Controlling for health status is imperative for survival studies examining psychological risk factors as health is a major confounder of the association between psychological risk factor and death. For example, people with poor health consistently have a lower level of positive affect and a higher degree of worry.(1) Other potential confounders, which should be taken into account when considering the association between positive affect and survival, are socio-economic status and lifestyle factors, as these are associated with survival(19-20) and positive affect.(8, 21) The association of positive affect with survival might also be confounded by negative affect as characterized by e.g fear, loneliness, and sadness. Negative affect is the key symptom of depression, which in turn is a risk factor for poor health and mortality. (22-24)

The availability of validated and objective measures of positive affect and covariates in the Rotterdam Study allowed us to study the association between positive affect and survival taking into consideration health status, negative affect, socioeconomic status and lifestyle factors as possible confounders. In addition we investigated possible age-dependence given that the association of positive affect with survival is suggested to depend on the age range studied.(1, 16)

Methods

Design and Case Ascertainment.

The current study was set within The Rotterdam Study, a population based cohort designed to examine the onset of disease in older adults. A more detailed description is provided elsewhere. (25)All participants aged 55 years and over in the Ommoord district of Rotterdam, The Netherlands were invited to participate (1990–1993). (26) All participants engaged in a home interview and a subsequent visit to the research centre for clinical assessments. During the third follow-up examination (1997-1999) screening with the Center for Epidemiological Studies Depression scale (CES-D), was added to the protocol. As such this examination was used as the baseline for the current study. In this survey 4,797 persons participated (79%), of which 4,411 completed the CES-D scale.

Positive and Negative Affect Measurement

The CES-D scale is a widely used standardized self-report instrument used for measuring current depressive symptoms and the identification of potential cases of depressive disorders. This measure has been validated in multiple populations.(27) It consists of 20 items indicating mood and feelings experienced in the past week. Responses are scored on a 4-point scale with descriptive anchor points: 0 – "Rarely or none of the time (0-1 day)", 1 – "Some or a little of the time (1-2 days)", 2 – "Occasionally or a moderate amount of the time (3-4 days)", and 3 – "Most or all of time (5-7 days)". A previous factor analysis of the CES-D showed that the scale has four underlying factors: interpersonal relations, somatic problems, negative affect and positive affect.(27-28) The positive and negative affect sub-scales have previously been used in population-based studies of mortality.(11, 13) Confirmatory factor analysis confirmed these four factors with a comparative fit index (CFI) of 0.967 and root mean square error of approximation (RMSEA) of 0.038. The positive affect factor consists of four items related to positive affect, these items are presented in Table 1. For the present analyses, the scores from the individual items were summed and used as a continuous variable, providing a potential score range from 0 to 12. The negative affect sub-scale consists of seven items (also presented in Table 1) and was used as a continuous variable with a score range from 0 to 21. Positive affect and negative affect were significantly correlated (p=0.00); the correlation coefficient between the affect scores was 0.63.

Mortality Assessment

Mortality was assessed through continuous monitoring of the municipal address files and computerized reports from general practitioners provided upon the death of a participant. Mortality follow-up was completed until January 1, 2007. From the group of 4,411 participants,

1,287 mortality events were observed, with a completeness of follow up of 99,11%. The mean observed number of person-years was 7.19 (SD=2.20).

Covariate Assessment

All covariates were assessed at baseline. Marital status was categorized into: single, married, widowed, divorced. Highest education achieved was recorded and categorized as: elementary, lower secondary, higher secondary, tertiary. Current occupational status was recorded as unemployed or employed. Smoking status was coded in categories as never, former and current smoker. Alcohol use was coded as never, former and current alcohol drinker. Height (m) and weight (kg) were measured and Body Mass Index (BMI) was calculated as kg/m^2 and used as a continuous variable. Current exercise level was coded as non-exerciser and exerciser. Disability status was assessed by calculating the Disability Index Score (29) from the validated Stanford Health Assessment Questionnaire (30) and categorised as: no disability (0-0.5) moderate disability (0.5-1) or severe disability (>1). Information on prevalent chronic disease at baseline (stroke, myocardial infarction, heart failure and several types of cancer – prostate, breast, colon, lung) was obtained from general practitioners files. Two research physicians coded events and in the case of disagreement a medical specialist was consulted. Variables for prevalent diseases were coded as 0 when participants never had the disease and 1 when participant had the disease in the past or have the disease at baseline. Systolic blood pressure of the right brachial artery was measured twice by a trained research assistant with a random-zero sphygmomanometer after the subjects had rested for 5-min. For analyses the mean of the two blood pressure measurements was calculated

Statistical Analyses

We used ANOVA for continuous/dichotomous variables and chi-square test for categorical variables to describe the associations of positive affect with the covariates. For this we used positive affect categorically with participants scoring low on positive affect (positive affect score 0-7), medium (positive affect score 8-11), high (positive affect score = 12).

The association between positive affect and survival was evaluated using Cox proportional hazards models. The proportional hazards assumption was assessed for all predictors using time dependent interaction terms. Cancer prevalence violated the proportional hazards assumption, hence we used heavyside functions(31) for this variable such that the survival follow-up time was stratified according to <5 years and >5 years.

Univariate, age and sex adjusted, and fully adjusted analyses were conducted. For the fully adjusted analyses covariates were clustered into different domains: affect, socioeconomic, lifestyle, and health status at baseline. Stepwise Cox regression analyses were conducted such that these covariate domains were added sequentially and cumulatively to the age and sex adjusted model to determine if positive affect remained associated with survival after controlling for each subsequent domain of variables. To asses a dose response relationship we used positive affect categorically as described above.

The positive affect by age interaction on survival was tested by separately adding a multiplicative term of these variables into the fully adjusted model. Because the estimate of the interaction term of positive affect with age on survival departed from a multiplicative effect, we stratified by 10-year age groups to illustrate the effect within age groups. The positive affect by health status interaction was tested by separately adding a multiplicative term of these variables into a fully adjusted model, including health status (dichotomous) instead of the separate variables concerning health status at baseline. Health status was defined as 0 when participants were free of any prevalent disease that is accounted for in the analysis and had no disabilities, and 1 when otherwise. In additional analyses we also stratified by health status.

Results

Baseline characteristics are presented in Table 2. The median age of the sample was 71.9 years with an age range of 61.2-102.4 years. Compared to low positive affect, participants with high positive affect were significantly younger (p=<0.01), more likely to be male (p=<0.01), higher educated (p=<0.01), more likely to be employed (p=0.007), more likely to be married (p=<0.01), less likely to smoke (p=<0.01), more likely to drink alcohol (p=<0.01). Moreover, persons with high positive affect scores were less likely to be disabled (p=<0.01), more likely to exercise (p=<0.01), and less likely to have baseline prevalence of stroke(p=<0.01) or heart failure(p=<0.01).

There were no significant differences in BMI, baseline prevalence of myocardial infarction or cancer, and systolic blood pressure.

Positive Affect and Survival

In the univariate analyses (Table 2) positive affect was a significant predictor of survival, such that a higher positive affect was associated with a lower risk of mortality (HR 0.93; 95% Confidence Interval (CI): 0.91,0.95; $p < 0.001$). The estimated effect of negative affect on survival was only significant when modeled univariately (HR1.05; 95% CI:1.03,1.07; $p <$

0.001). When positive affect was added to the analysis the estimated effect of negative affect was no longer significant (HR 1.01; 95%-CI:0.98,1.03).

Age and sex adjusted and multivariate analyses are presented in Table 3. Adjusting for age and sex had little effect on the association between positive affect and survival (HR 0.95; 95% CI 0.93,0.97; p < 0.001). A similar finding was present for the first step of the adjusted model which added socioeconomic variables. When lifestyle variables were added in the next step of the adjusted model, the association of positive affect with survival was attenuated but remained significant (HR 0.97; 95% CI 0.95,0.99; *p*= 0.01). However, when health status was entered in the final step of the adjusted model positive affect no longer predicted survival (HR: 0.98; 95% CI 0.96,1.01; *p* =0.13). In the fully-adjusted model, in addition to age and sex, smoking status, exercise, alcohol usage, prevalence of myocardial infarction, stroke, dementia, heart failure and cancer were all significantly associated with survival.

Interaction of Positive Affect with Age and Health status

We observed a significant interaction of positive affect with age in the fully adjusted model (*p*-value interaction term fully adjusted model=0.02). Subsequent age stratification was conducted to determine if there was a differential association between positive affect and survival within each age strata, results are presented in Table 4. Age and sex adjusted models revealed that positive affect was associated with survival in each age group strata. For adults aged under 70 years positive affect remained a significant predictor of survival even fully adjusted (HR 0.92; 95%-CI:0.86,0.98; *p* = 0.01). In this association, there was a dose-response relationship between positive affect and mortality. In the younger old the HR of a medium score for mortality was 0.72 (95%-CI:0.45,1.13) compared to those scoring low. Participants scoring high had an HR for mortality of 0.59 (95%-CI:0.37,0.92) in the fully adjusted analyses (age, sex, negative affect, socioeconomic status, lifestyle and baseline health status). For adults aged 70-80 years positive affect was a significant predictor of survival after controlling for negative affect, socioeconomic status and lifestyle, but the addition of health status to this model rendered the association insignificant (HR 0.97; 95%-CI:0.94,1.07; *p* = 0.11). For adults aged >80 years positive affect did not predict survival after controlling for negative effect, socioeconomic status and lifestyle factors (HR 0.97; 95%-CI:0.94,1.01; *p* =0.16).

To further illustrate and investigate this association we also dichotomized the three age strata, contrasting the oldest-old at baseline (>80 years) with the middle and younger-old persons (<80 years). Positive affect was significantly associated with survival after controlling for all covariates in the younger and middle-old persons (<80 years of age: HR 0.96; 95%-

CI:0.93,0.99; p =0.01), whereas in the oldest-old persons the association was not significant after adjustment for covariates (HR 1.00; 95%-CI:0.96,1.04; p-value=0.79).

Positive affect did not significantly interact with health status on survival (p-value interaction term=0.61) in a model including age, sex, negative affect, socioeconomic variables lifestyle variables, and health status (dichotomous). When we additionally stratified by health status, the fully adjusted association of positive affect with survival was not statistically significant in any of the six strata. In participants healthy at baseline, the HR of positive affect on survival in the younger old was 0.94 (95%-CI;0.85,1.04), and in the middle old 0.96 (95% CI:0.89,1.04). In the small group of oldest old, the point estimate was (HR 1.06; 95%-CI:0.94,1.20). In participants with a prevalent disease or disabilities at baseline, the HR of positive affect on survival was 0.93 (95%-CI:0.86,1.01) in the younger old, 0.90 (95%-CI:0.95,1.03) in the middle old and 0.96 (95%-CI:0.92,1.00) in the oldest old.

Discussion

Positive affect was found to predict survival in older adults in univariate analyses, such that a higher level of positive affect provides a protective effect against survival. This association remained after controlling for age, sex, negative affect, socioeconomic status and lifestyle factors but was not significant anymore after controlling for health status. However, we did find a significant age dependent association of positive affect with survival. For the younger old adults a more positive affect was related to reduce mortality while in the oldest-old persons positive affect was not associated with survival after adjusting for socio-economic status, life-style and health.

Several previous studies reported a better survival of persons with more positive affect.(1, 8-9, 12-14, 17, 32-33) Positive affect was associated with survival in a population based study in 2,282 older individuals by Ostir et al(13), even after controlling for age, sex, baseline medical conditions, body mass index, smoking, drinking, sociodemographics and negative affect. However this study had a limited follow up time of 2 years. A smaller study of 660 older individuals with 23 years of follow up by Levy et al.(32) also reported positive affect to be associated with survival after controlling for age, sex, socioeconomic status, and baseline health status. Interestingly, a large study including 6,928 individuals with a wide age range (16-94) by Kaplan and Camacho(16), observed no association between happiness and survival after 9 years of follow up. Although they did not study age interaction this may suggest that the association of positive affect with survival is inconsistent across age ranges. In addition some evidence for an age interaction in elderly persons has been reported in a small study of 421

individuals by Parker et al.(15) Here life satisfaction was significantly associated with survival in the younger old (75-85) but not in the older old(<85). Our results also suggest that the association of positive affect with survival is inconsistent across age ranges and are based on a large sample, considerate follow up time, and controlled for multiple well assessed confounders. This suggests that not only control for confounders but age range accounts for inconsistencies in published results.

We additionally checked whether the association of positive affect with survival was modified by health status. However, it is likely this analysis is underpowered. For example, only 175 persons (85 cases) very old persons with a mean follow up of 6.94 years contributed person-time to the healthy stratum, all results should therefore cautiously be interpreted.

In general, several mechanisms have been suggested to explain the association of positive affect with survival. One potential mechanism is better health behavior.(34) People with a higher positive affect are more inclined to watch their weight, are more perceptive of symptoms of illness, engage more often in sport and tend to be moderate with smoking and drinking.(8) It is also been suggested that people with higher positive affect make better health relevant choices in life because they are more open to the world and are more self-confident.(35) Therefore, people with a higher positive affect may live longer but this may not directly be due to positive affect, but due to a healthy lifestyle and protective health behavior. Although we can not exclude this possibility in the present study, we were able to adjust for some of these factors, such as BMI, exercise, smoking status and alcohol use to limit confounding because of lifestyle and health behavior. Another possible mechanism is a direct effect of positive affect, possibly through favorable biological responses, including low cortisol levels, faster cardiovascular stress recovery and reduced inflammation resilience to infection.(2, 34, 36-39) Why increased levels of positive affect might be beneficial in the younger old in particular, requires further research. We adjusted for health status as measured by several variables, but this may mean that we corrected for the consequences of positive affect in younger years. To some extent good health may be the result of positive affect. On the other hand, prior research suggested that life satisfaction and quality of life in the oldest-old is strongly determined by absence or presence of chronic diseases.(40) Herefore we posit that the perception of happiness in the oldest old is largely determined by health status.

As several mechanisms could explain the association of positive affect with survival, covariate selection for the analysis was especially important. One other study reported an association between positive affect and survival did not report the association upon adjustment for socio-demographic variables and perceived health.(17) Disregarding age dependence our

fully adjusted model also suggests that positive affect was not associated with survival if the effects of other variables are accounted for. However, the inclusion of some variables in our fully adjusted model may be considered as over-adjustment. “Over-adjustment” refers to the inclusion of mediators of the association between happiness and survival in the model.(41) Our study population consists of older people and it is plausible that prior happiness determined baseline health, and so any assessment of baseline health could reflect the consequences of prior positive affect. With the current design it is not possible to completely solve this issue. Hence we can only reason the most likely causal pathway.(42) We argue that the covariates in the present study are independently associated with survival, and are not, or only to a small extent, mediators of the association between positive affect and survival.

A different observation was on the limited impact of negative affect on survival. In the present analysis negative affect was inversely associated with survival only in the univariate analysis, in the younger old. In contrast, positive affect was a predictor of survival even after adjustment for negative affect, in some analyses. Therefore our results suggest that the positive affect component in the CES-D scale is a more powerful predictor of survival than negative affect. This is in line with other survival studies. Although, many studies report either positive affect or negative affect, few studies, analyzed both positive and negative affect. One study in 4,162 community-dwelling elders showed that only positive affect was a predictor of survival not the other factors of the CES-D scale, independent of age, sex, race, civil status, education, cognitive impairment and functional impairment. (10) Further, a survival study of 2,282 Mexican Americans showed that positive affect was a predictor of survival even independent of negative affect along with age, socio-demographic variables, prevalence of some chronic diseases.(13) Finally, a study on cytokines reported that positive affect, not negative affect, was associated to increased infection-induced pro-inflammatory cytokines and thereby resilience to infections.(39) This is interesting since this suggests that positive affect and not negative affect has beneficial health effects, while negative affect is considered to be the key symptom of depressive disorder which in itself is a major risk factor for health risks and survival.(22-24)

The present study has several strengths, such as the sample size and the population based approach utilized. Further, the current analyses considered multiple confounders related to negative affect, life style, socioeconomics, and health that were assessed by trained interviewers or clinicians, and in case of prevalent disease status adjudicated through revision of medical records. On the other hand this study is based on a single measurement of positive affect, derived from the CES-D scale. Single measurements are criticized on the grounds that they are

affected by recall bias, and the dominant influences of current state.(37) However, in older adults positive affect is considered a stable characteristic that is unlikely to vary over time.(43)

In conclusion, the current study suggests that the association of positive affect with survival is not consistent over the age range studied. In the younger old, positive affect did significantly predict survival independent of negative affect and health. In the oldest-old the association of positive affect to survival might be moderated by differences in health status.

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Table 1. CES-D Scale items for positive and negative affect

Positive Affect:
• I felt that I was just as good as other people
• I felt hopeful about the future
• I was happy
• I enjoyed life
Negative Affect:
• I felt that I could not shake off the blues even with the help of my family or friends
• I felt depressed
• I thought my life had been a failure
• I felt fearful
• I felt lonely
• I had crying spells
• I felt sad

Table 2. Baseline Characteristics of Study Population and Univariate Cox Regression Predicting Survival (*n*=4411)

Variable	Descriptive Statistics	Univariate Model	
	n (%) / Mean (SD)	HR	(95% CI)
General			
Sex			
Male	1795 (40.7%)	1.00	(Reference)
Female	2616 (59.3%)	0.71	(0.63, 0.80)
Age	72.74 (7.28)	1.11	(1.10, 1.12)
Affect			
Positive affect †	10.3 (2.62)	0.93	(0.91, 0.95)
Negative affect †	2.8 (4.85)	1.05	(1.03, 1.07)
Socioeconomic			
Employment			
Unemployed	4216 (95.6%)	1.00	(Reference)
Employed	195 (4.4%)	0.39	(0.26, 0.58)
Marital status			
Married	2819 (63.9%)	1.00	(Reference)
Single	246 (5.6%)	1.20	(0.94, 1.52)
Widowed	1127 (25.5%)	1.82	(1.62, 2.05)
Divorced	219 (5.0%)	1.00	(0.76, 1.32)
Education			
Elementary	2137 (48.4%)	1.00	(Reference)
Lower secondary	742 (16.8%)	0.80	(0.68, 0.94)
Higher Secondary	1144 (25.9%)	0.85	(0.75, 0.98)
Tertiary	388 (8.8%)	0.72	(0.57, 0.89)
Lifestyle			
Exercise			
Non-exerciser	2947 (66.8%)	1.00	(Reference)
Exerciser	1464 (33.2%)	0.56	(0.49, 0.64)
Smoker			
Never	1529 (34.7%)	1.00	(Reference)
Past	2161 (49.0%)	0.95	(0.84, 1.07)
Current	721 (16.3%)	1.23	(1.05, 1.43)
Alcohol Use			

Never	503 (11.4%)	1.00	(Reference)
Past	344 (7.8%)	1.51	(1.21, 1.87)
Current	3564 (80.8%)	0.81	(0.69, 0.96)
Body Mass Index (kg/m^2)	26.82 (3.97)	0.97	(0.96, 0.99)
Health Status			
Disability Status			
None	2937 (66.6%)	1.00	(Reference)
Moderate	748 (17.0%)	1.90	(1.64, 2.19)
High	726 (16.5%)	3.76	(3.31, 4.26)
Prevalent Disease			
Myocardial infarction	512 (11.6%)	1.86	(1.61, 2.15)
Heart failure	261 (5.9%)	3.37	(2.86, 3.97)
Stroke	196 (4.4%)	2.66	(2.19, 3.23)
Dementia	172 (3.9%)	2.96	(2.42, 3.62)
Cancer	244 (5.5%)	1.86	(1.53, 2.27)
Systolic Blood Pressure (mm Hg)	143.5 (21.30)	1.01	(1.00, 1.01)

Note.

† For positive affect and negative affect the HR is expressed per unit change on CES-

Table 3. Stepwise Cox Regression Models Predicting Survival (n=4411)

	Baseline Model[a]			**+ Negative affect[b]**			**+ Socioeconomic[b]**			**+Lifestyle[b]**			**+ Health Status[b]**		
Covariates	HR	(95% CI)	*p*-value	HR	(95% CI)	*p*-value	HR	(95% CI)	*p*-value	HR	(95% CI)	*p*-value	HR	(95% CI)	*p*-value
General															
Sex															
Male	1.00	(Reference)		1.00	(Reference)		1.00	(Reference)		1.00	(Reference)		1.00	(Reference)	
Female	0.55	(0.49,0.62)	<0.01	0.55	(0.49,0.61)	<0.01	0.52	(0.46,0.59)	<0.01	0.54	(0.47,0.62)	<0.01	0.53	(0.46,0.61)	<0.01
Age	1.12	(1.11,1.12)	<0.01	1.11	(1.11,1.12)	<0.01	1.11	(1.10,1.12)	<0.01	1.11	(1.10,1.12)	<0.01	1.09	(1.08,1.10)	<0.01
Affect															
Positive Affect †	**0.95**	**(0.93,0.97)**	**<0.01**	**0.96**	**(0.93,0.98)**	**<0.01**	**0.96**	**(0.93,0.98)**	**<0.01**	**0.97**	**(0.95,0.99)**	**0.01**	**0.98**	**(0.96,1.01)**	**0.13**
Negative Affect †				1.01	(0.98,1.03)	0.51	1.01	(0.98,1.03)	0.70	1.01	(0.98,1.03)	0.63	0.99	(0.97,1.02)	0.66
Socioeconomic stats															
Employment															
Unemployed							1.00	(Reference)		1.00	(Reference)		1.00	(Reference)	
Employed							0.75	(0.50,1.13)	0.17	0.76	(0.51,1.15)	0.19	0.80	(0.53,1.21)	0.29
Marital status															
Married							1.00	(Reference)		1.00	(Reference)		1.00	(Reference)	
Single							0.83	(0.58,1.18)	0.30	0.80	(0.56,1.15)	0.22	0.85	(0.66,1.09)	0.19
Widowed							0.91	(0.68,1.21)	0.51	0.95	(0.71,1.26)	0.73	1.05	(0.91,1.22)	0.48
Divorced							1.02	(0.76,1.36)	0.90	1.01	(0.76,1.35)	0.93	1.01	(0.76,1.34)	0.95
Education															
Elementary							1.00	(Reference)		1.00	(Reference)		1.00	(Reference)	
Lower secondary							1.12	(0.90,1.41)	0.32	1.04	(0.82,1.30)	0.77	1.00	(0.85,1.18)	0.96

Higher secondary	1.06	(0.82,1.38)	0.64	1.00	(0.77,1.29)	1.00	0.99	(0.86,1.14)	0.91
Tertiary	1.08	(0.85,1.38)	0.51	1.05	(0.83,1.34)	0.67	0.99	(0.78,1.25)	0.92
Lifestyle									
Exercise									
Non-Exerciser				1.00	(Reference)		1.00	(Reference)	
Exerciser				0.70	(0.62,0.80)	<0.01	0.75	(0.66,0.86)	<0.01
Smoker									
Never				1.00	(Reference)		1.00	(Reference)	
Past				0.57	(0.48,0.68)	<0.01	1.04	(0.90,1.20)	0.60
Current				0.58	(0.50,0.68)	<0.01	1.72	(1.44,2.04)	<0.01
Alcohol use									
Never				1.00	(Reference)		1.00	(Reference)	
Past				1.04	(0.87,1.23)	0.69	1.28	(1.02,1.60)	0.03
Current				1.30	(1.09,1.55)	<0.01	1.10	(.92,1.31)	0.31
Body Mass Index kg/m^2				0.99	(0.97,1.01)	0.16	0.98	(0.96,0.99)	<0.01
Health Status									
Disability Status									
None							1.00	(Reference)	
Moderate							1.52	(13.28,1.74)	<0.01
High							2.62	(2.16,3.19)	<0.01
Prevalent Disease									
Myocardial Infarction							1.23	(1.06,1.44)	0.01
Heart failure							1.58	(1.33,1.88)	<0.01
Stroke							1.54	(1.26,1.89)	<0.01
Dementia							1.92	(1.55,2.37)	<0.01
Cancer									

<5 years follow up	13.87	(10.26,18.75)	<0.01
>5 years follow up	0.79	(0.60,1.04)	0.09
Systolic blood pressure (mm Hg)	1.00	(1.00,1.00)	0.61

Note.

[a] The baseline model is adjusted for sex, age and positive affect.

[b] In addition to all variables included in previous models.

† For positive affect and negative affect the HR is expressed per unit change on CES-D.

Table 4. Cox Regression Analyses of Positive Affect and survival within Age Group Strata *(n=4411)*

		Model 1[a]			Model 2[b]			Model 3[c]		
	N	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age continuous										
Positive affect†	4411	0.89	(0.84 , 0.95)	<0.01	0.91	(0.85 , 0.97)	<0.01	0.92	(0.86 , 0.98)	0.01
Age	4411	2.28	(1.74 , 2.99)	<0.01	2.24	(1.70 , 2.96)	<0.01	1.73	(1.31 , 2.28)	<0.01
Positive affect * Age	-	1.03	(1.00 , 1.06)	0.03	1.03	(1.00 , 1.06)	0.03	1.03	(1.00 , 1.06)	0.02
Age Group Strata										
Positive affect , <70 years	1798	0.90	(0.86 , 0.94)	<0.01	0.91	(0.86 , 0.97)	<0.01	0.92	(0.86 , 0.98)	0.01
Positive affect, 70-80 years	1866	0.95	(0.92 , 0.98)	<0.01	0.96	(0.93 , 1.00)	0.04	0.97	(0.94 , 1.07)	0.11
Positive affect, >80 years	747	0.96	(0.93 , 0.99)	0.01	0.97	(0.94 , 1.01)	0.16	1.00	(0.96 , 1.04)	0.79

Note.

[a] Adjusted for Age and Sex.

[b] Adjusted for age, sex, negative affect, lifestyle and socioeconomic factors.

[c] Adjusted for age, sex, negative affect, lifestyle, socioeconomic and baseline health status.

† For positive affect the HR is expressed per unit change on CES-D.

4. Prediction of Mortality

CHAPTER 4.1

Genetic, Physiological and Lifestyle Predictors of Mortality in the General Population

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ABSTRACT

Objective

We investigated the quality of 162 variables focusing particularly on the contribution of genetic markers, used solely or in combination with other characteristics, when predicting mortality.

Methods

In 5974 participants from the Rotterdam Study, followed for a median of 15.1 years, seven groups including age and gender, genetics, socio-economics, lifestyle, physiologic characteristics, prevalent diseases, and indicators of general health were related to all-cause mortality. Genetic variables were extracted from genome-wide association scans of eight discovery cohorts (n = 19,033) and identified by literature review.

Results

We observed 3174 deaths during follow-up. The fully adjusted model (C-statistic for 15 year follow-up (C_{15y}): 0.80, 95% CI: 0.75-0.77) predicted mortality well. Most of the information beyond age and sex stemmed from physiologic markers, prevalent diseases, and general health. Socio-economic factors and lifestyle contributed meaningfully to mortality risk prediction with longer prediction horizon. Although specific genetic factors were independently associated with mortality, jointly they contributed little to the prediction of mortality (C_{15y}: 0.56, 95% CI: 0.55-0.57). Unlike other characteristics the predictive ability of genetic markers remained constant during follow-up.

Conclusion

Mortality can be predicted reasonably well even over a long period. Genetic factors independently predict mortality but their contribution above other risk indicators is modest.
Keywords: mortality, survival analysis, gender, GWA, genes, prediction

INTRODUCTION

In the 20th century life expectancy at birth increased from 50 years to over 80 years in Western countries.[1] Demographers repeatedly predicted that it had reached a ceiling, but life expectancy in record countries continues to rise by an average of three months each year.[2] Although epidemiological research identified numerous predictors of mortality, information about their comparative effect sizes and long-term predictive power is sparse. Prior research has often been limited by a short period of follow-up, a limited set of covariates or by focusing on cause-specific mortality. Only a few studies have evaluated the potential for explaining mortality from a broader perspective by jointly analyzing demographic characteristics, lifestyle factors and indicators of health and disease.[3-7] It is still unclear whether genetic information can be used to predict mortality, but recent advances in genomic technology allow for the inclusion of genetic markers in the prediction of mortality.

In the present study, we combine traditional indicators of mortality risk with genetic factors, derived from a meta-analysis of eight genome-wide association studies and the literature, and associate them with mortality over 15 years of follow-up. Our aims are twofold: firstly, to identify independent determinants of mortality by analyzing 162 a priori identified risk factors; secondly, to inform about the independent and combined potential of genetic markers in predicting mortality.

METHODS

Study Population

The Rotterdam Study is a population-based prospective cohort initiated in 1990 designed to investigate risk factors for diseases in 7983 participants aged 55 years or over in the Ommoord district of Rotterdam, The Netherlands.[8-9] In the initial and subsequent investigation waves trained research assistants collected data on health, medication use, medical and family history, and lifestyle factors in extensive home interviews. Participants subsequently visited the research center for clinical examinations with a special emphasis on imaging and on collecting and storing biospecimens to facilitate in-depth molecular and genetic analyses. Data analyzed in this study concern 5974 participants with genetic information available from the first wave of the Rotterdam Study. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center. All participants provided written informed consent.

Predictors

We organized baseline data into related groups: age and gender, genetics, socio-economics, lifestyle, physiology, diseases, and general health. We hypothesized a priori that genetics, socio-economics and lifestyle were associated with long term health effects, while physiology, disease, and general health were more likely associated to short term mortality.[10] Overall we analyzed 162 risk indicators in this study; 69 previously studied risk factors for mortality and 93 single nucleotide polymorphisms (SNPs).

To study possible genetic risk factors of mortality, we used genetic data of 19,033 participants (55% women) aged 55 years and older from eight discovery cohorts of European ancestry, participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), other than the Rotterdam Study.[11] We identified the top 50 loci from the meta-analysis of genome-wide association studies (GWAS) on time to death. In addition we used 43 SNPs that mapped to genes from a seminal review[1]. For the analysis, all SNPs were extracted from the imputed gene information of the Rotterdam Study, except for Apolipoprotein E (ApoE) which was genotyped directly.[12] Information about cohort characteristics, genotyping, and imputation of the discovery set is presented in Table 1 and 2 in the Supplementary Material.

The following variables describing the socio-economic status of the study population were included: education, employment status, monthly income, social class (derived from occupation of head of the household), health insurance status, number of children, living independently or in a nursing home, living with a partner, and death of spouse.[8-9]

We included the following indicators of lifestyle: riding a bike, alcohol consumption, smoking, energy intake, and fruit and vegetable consumption.[13-14]

Physiology was assessed as: body weight, body mass index, waist circumference, hip circumference, waist-to-hip ratio, sitting systolic and diastolic blood pressures, leukocyte count, erythrocyte sedimentation rate, albumin level, total cholesterol level, high-density lipoprotein cholesterol, creatinine, uric acid, serum C-reactive protein (CRP), postload insulin, bone mineral density of the femoral neck and lumbar spine, and atherosclerotic plaques. All physiologic variables were assessed using standard medical, laboratory, and imaging procedures as described previously.[15-20]

Based on self report, investigations at the baseline center visit, medical record information, and drug utilisation we defined the following prevalent diseases: diabetes, left ventricular hypertrophy, atrial fibrillation, hypertension, hip fracture, peripheral artery disease, myocardial infarction, heart failure, dementia, gout, Parkinson's disease, stroke,

transient ischemic attack, cancer, cognitive function (minimental state examination, MMSE), and coronary operation.[21-27]

General health included: Activities of Daily Living[28] and Instrumental Activities of Daily Living[29], health care utilization, self-perceived comparative health, accidental falls, shortness of breath, past serious illness and hospitalization, unintentional weight loss, and self-reported memory complaints.

Outcome

The outcome measure was time to death from any cause. All participants of the Rotterdam Study were under continuous surveillance; general practitioners' and hospital records as well as death certificates from the municipality to identify deceased participants until January 1, 2009. The median follow-up was 15.1 years (range, 0.05 to 19.50).

Statistical Analysis

We used SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and the PROC MI procedure to impute to 5 complete data sets. The maximum missingness for analysis of the data was set to 30% *a priori* and the percentage of missing information is reported in the supplementary material Table 3. Other than age, all continuous variables were standardized to facilitate the comparison of effect sizes. The estimates represent the effect of a change of one standard deviation (SD).

We analyzed the risk indicators and their association with mortality with Cox proportional hazard models. We calculated unadjusted hazard ratios (HR) and confidence intervals (CI) of each, and subsequently optimized the predefined groups separately, adjusting for age and gender within each group, by means of stacked imputed backward regression with p=0.2 as exclusion threshold.[30] Finally, we combined all remaining variables in a final model using backward regression. We also used least angle absolute shrinkage operator (LASSO) penalized regression as implemented in the R package "penalized" to validate the results from backward regressions.[31-32] The proportional hazard assumption was evaluated using Schoenfeld residuals.[33] Variables that did not fulfill the proportional hazards assumption in the imputed datasets were modeled with piecewise constant Heaviside functions.[34] We considered $p < 0.05$ from two-sided tests as statistically significant.

Time-dependent receiver-operating characteristics (ROC) curves were used to compare the predictive performance of the different variable groups over time.[35] ROC curves describe the relationship between sensitivity (true positive rate) and 1 – specificity (false positive rate) for all possible cut-offs of a marker to distinguish between high risk participants and low risk participants. We also computed the C-Index, the probability that a subject who

dies on any given day during a specified time interval has a higher predictive score than a subject who survives beyond that day. For this part of the analysis, we accounted for residual time dependency using Schoenfeld smoothing.[35] Confidence intervals were estimated using cross-validation in 500 bootstrap samples.

RESULTS

At baseline, participants were on average 69 years of age (range: 55 – 99 years, Table 1) and 59% were female. Of the 5,974 participants 3,174 died (mortality rate: 4.2 per 100 person-years) during the follow-up period. From 162 a priori identified risk factors of mortality shown in Table 3 of the Supplementary Material, backward regression retained 108 variables independently in the final model. Of these, 36 were significantly related to mortality ($p < 0.05$) as independent risk factors (Table 1).

Age (HR, 95%-CI: 1.09, 1.08-1.10) and female gender (HR: 0.71, 0.62-0.81) were strongly associated with mortality (Table 1).

From the candidate genes, *a priori* identified in the literature, *ApoE,* Insulin-like growth factor 1 Receptor (*IGF1R*), and Werner syndrome, RecQ helicase-like (*WRN*) were significant and independent predictors of mortality. From the 50 independent GWAS loci, identified from the meta-analysis of eight discovery cohorts on time to death, two SNPs in the neighbourhood of tripartite motif-containing 32 (*TRIM32*) and methionine adenosyltransferase II, beta (*MAT2B*) were associated with mortality.

Social class and living in serviced housing were independently associated with risk of death. Smoking status and pack-years as well as energy intake were also associated with mortality. The physiologic measures blood pressure, body mass index, waist circumference, and in particular the risk indicators assessed in blood, such as erythrocyte sedimentation rate, leucocytes, creatinine, C-reactive protein, total cholesterol, or with imaging, such as bone mineral density of the femoral neck and aortic calcification, were all independently related to mortality. Diabetes, cardiac diseases, Parkinson, cancer, and cognitive function remained independently associated with death. Self perceived, comparative health was a good indicator of mortality risk, as were unintentional weight loss and serious illness in the past 5 years.

The predictive power of the variable groups is best explained in two ways. First, Figures 1 and 2 show the development of predictive quality over time. For each point during follow-up, the graphs depict the respective time-dependent area under the curve (AUC) of a given variable group. Next, Table 2 quantifies the predictive quality for five specific prediction intervals (1, 3, 5, 10, and 15 years).

Figure 1 shows that, over time, all variable groups except genetic risk markers exhibited decreasing ability to predict death. Figure 1 and Table 2 also demonstrate that prediction based solely on age and gender consistently outperformed all other groups of variables (C_{15y}: 0.76, 95% CI: 0.75-0.77, see Table 2). Physiological risk and socio-economic characteristics each predicted mortality equally well over 15 years (each C_{15y}: 0.72, 95% CI: 0.71-0.73). Although of significantly less predictive quality, the C-index of genetic risk markers was still better than chance (C_{15y}: 0.56, 95% CI: 0.55-0.57).

Figure 2 shows the performance of the age-and-gender adjusted model compared to the fully adjusted model (C_{15y}: 0.80, 95% CI 0.79-0.81). Whereas adding socio-economic and lifestyle information to age and gender (C_{15y} : 0.77, 95% CI: 0.77-0.78) only slightly improved the predictive quality, the combination of age, gender, general health, disease, and physiology (C_{15y}: 0.79, 95% CI: 0.78-0.80) predicted mortality almost as well as all covariates that remained after backward regression.

To allow comparison to other studies of cause specific mortality and different population health status we report the associations of the final model stratified by prevalent, baseline disease status and for cardiovascular disease mortality in the supplementary material Table 4.

DISCUSSION

From a set of 162 established risk factors and candidate SNPs for mortality, we identified 36 (31 non-genetic, 5 genetic) independent and significant predictors of mortality. Specific genetic factors were independently associated with mortality and jointly predicted mortality better than chance. However, genetic information added little to age, gender and other traditional predictors of mortality.

This analysis confirms prior findings that multiple diseases, as well as socio-economics and lifestyle jointly influence mortality in the ageing adult population.[3, 6] Numerous predictors remained independently and significantly associated with mortality. While several markers of prevalent disease remained associated with mortality, their risk ratios were attenuated. Others such as prevalent dementia, cerebrovascular accidents, and transient ischemic attacks did not predict mortality beyond indicators of disease severity and subclinical disease such as MMSE and CRP.

Several specific SNPs were independently associated with mortality in our analysis. In accordance with the literature, each additional copy of the *ApoE* ε4 allele increased mortality in our study cohort.[1, 36-37] Similarly *IGF1R* and *WRN* have been described before as being associated with longevity and ageing via improving stress resistance, innate immunity, metabolic maintenance and repair of DNA respectively.[1] This study also confirmed two novel loci in vicinity of *TRIM32* and *MAT2B* genes as being associated with death. These two loci were identified from the pool of SNPs identified by the meta-analysis of the GWAs discovery cohorts. These genes have recently been associated with cancer proliferation.[38-39]

Interestingly, unlike the predictive ability of all other domains, the ability of genetic markers to predict death remained constant during the course of follow-up. The stability of the predictive power of these SNPs observed in this study is probably due to the permanent nature of the genetic make-up. Other variables showed decreasing predictive power over time which can be explained by changes in the value of these variables during the course of follow-up.

Our results support the view that specific SNPs can be identified that are associated with mortality and might be used for risk prediction.[40-41] But the results also show that these common SNPs have very limited predictive power and that, especially when used in combination with traditional risk factors, they contribute very little, if anything at all, to improve the prediction of death in the general population 55 years and older.

Another finding relates to age and gender as predictors of mortality. In our study the relative risk for mortality per year of age in the univariate model was only reduced by 25% in

the fully adjusted model. This is compatible with the idea that ageing is not merely the clinical manifestation of disease but an underlying, disease independent, accumulation of pathophysiological changes that favour mortality over time.[42-44]

The gender differences in this study were not due to differences in prevalent diseases at the onset of the study. Females exhibited even stronger reduced risks after adjustment for other risk factors. This strongly suggests that the gender difference in survival cannot be explained by differences in health behavior and disease at baseline.[45] We can only speculate that the survival benefits of females can be found in gender effects or different genetic origins not accounted for in this analysis. One of the potential genetic candidates that could contribute to the female survival advantage is the X chromosome. [46] Another genetic candidate is mitochondrial DNA (mtDNA). It has been suggested that a possible intergenomic conflict between mtDNA and nuclear DNA favors female survival due to stronger selection in females because of maternal inheritance of mtDNA.[47] We could not include markers on the X chromosome and mtDNA because the X chromosome is not commonly analyzed in all the discovery cohorts and mtDNA is also not available on all genotyping platforms.

Prediction of mortality by age and gender improved only by 5% upon inclusion of all independent mortality predictors over the entire 15 years of follow-up. This is particularly interesting considering the multitude of independent risk factors identified in this study; and because all groups contributed significant variables. From the time-dependent AUC analyses two observations are particularly noteworthy: first, most of the additional information beyond age and gender stemmed from indicators of physiology, disease, and general health. Second, although socio-economic factors were equally good in predicting mortality as indicators of disease, the combination of socio-economics, life style, and genetic markers only contributed visibly to the explanation of mortality risk after 10 years of follow-up and beyond. Thus, although socio-economics and lifestyle were associated to mortality, they seemingly exerted their effects on mortality through physiologic risk indicators and disease rather than acting independently on mortality.[48] This underscores the importance of socio-economics and living conditions for public policy aimed at reducing health inequalities.

Summarizing the findings reported here and in other studies, one can insinuate a cascade from gene to individual health to death, in which every step is accompanied by environmental influences, some of which are controlled by the individual, such as physical activity and obesity, other defined by the individual´s living circumstances, cultural heritage and surroundings. Figure 3 illustrates at which stage during the course of ageing different

interventions, e.g. improvements in living circumstances or the introduction of a new therapeutic drug, can feasibly act and which health gains could be expected.

Limitations

The interpretation of this study asks for a cautionary note. It was not our aim to evaluate the size of the mortality risk associated with single risk factors. Several of the markers in this analysis describe the same underlying construct (e.g. body composition). Therefore the specific relative risks must be interpreted cautiously. Other important aspects of health such as physical activity and mental health are barely represented among the risk factors analyzed as only "riding a bike" and "self perceived comparative health" were available to approximate these important dimensions of health. Another limitation concerns the genetic markers used in this study. We only included autosomal SNPs. Genetic risk is transferred through several other mechanisms including DNA methylation, copy number variations, and mitochondrial DNA. Furthermore it is unlikely that this study can be used for constructing a risk score as this study has not been replicated externally. We did not seek external replication because of the multitude of specific risk factors and instead relied on bootstrapping and cross-validation for guiding the LASSO analysis and the estimation of the C-index. At the same time, the selection of SNPs is among the strength of this study, as the SNPs were identified from two sources, independent of the population under study. Other strengths are related to the multitude of risk factors and the prospective design with long follow-up.

Conclusion

We found 36 variables that independently and significantly predicted mortality in the Rotterdam Study population. Adding further risk indicators to age and gender improved our ability to predict death but the gain in predictive quality was modest, particularly in the long run. Surprisingly, specific genetic risk factors independently and as a group predicted mortality, but their added value to conventional predictors of mortality was low. Our findings also support the importance of primary prevention in the areas of socio-economics and life style as we could illustrate how these risk factors continuously influence mortality risk through their impact on physiologic risk status and disease as well as independently.

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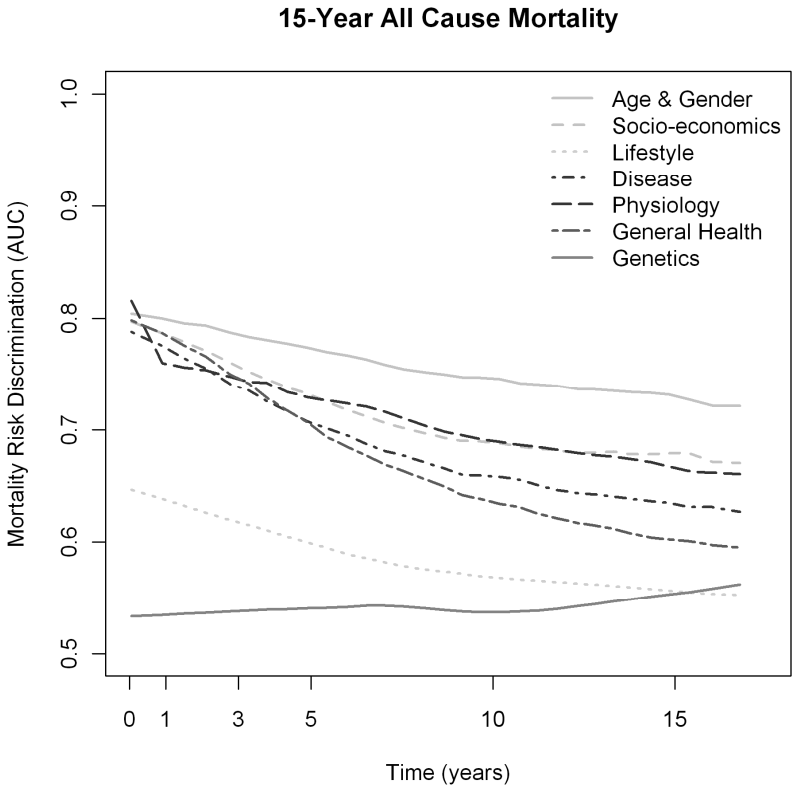

Figure 1 Time dependent ROC curves for different groups of variables.

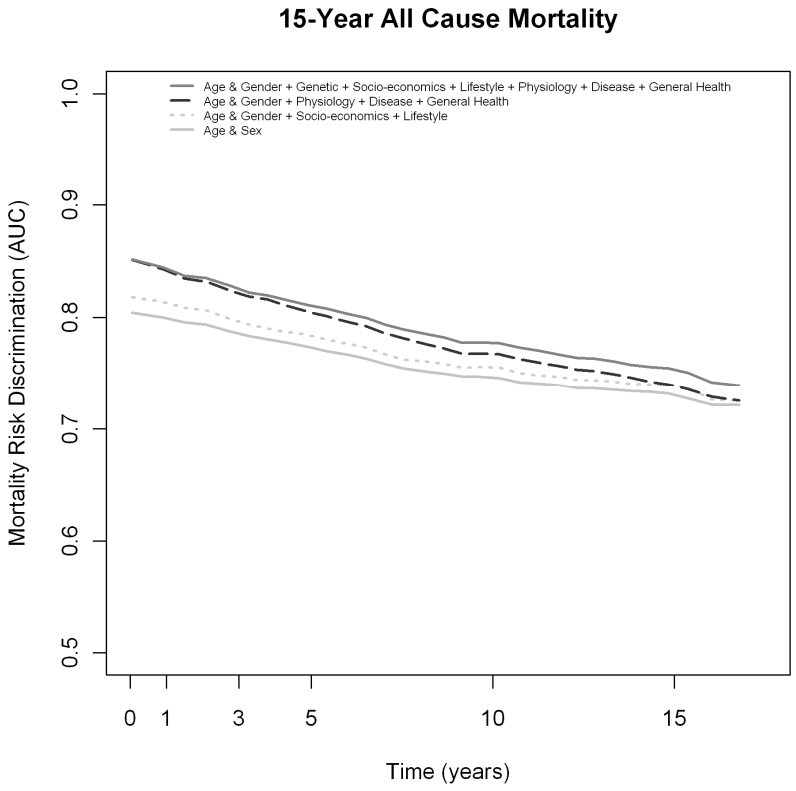

Figure 2 Time dependent ROC curves for age and gender and differently adjusted models

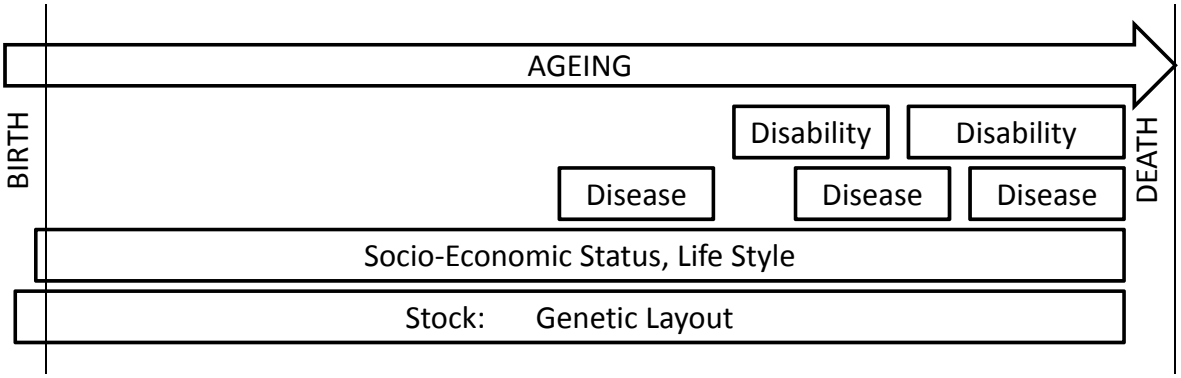

Figure 3 Mortality, ageing and the different groups of determinants that influence each other and jointly the individual's trajectory towards death.

Table 1 Descriptive statistics and association to 15-Year Mortality in 5974 participants from the Rotterdam Study: unadjusted and completely adjusted models*

	Baseline	Univariate		Final Model	
Variable	**mean (SD)/n (%)**	**RR (95%-CI)**	**p**	**RR (95%-CI)**	**p**
Age	69.43 (9.10)	1.12 (1.11-1.12)	<0.001	1.09 (1.08-1.10)	<0.001
Gender (female)	3547 (59.38)	0.79 (0.73-0.85)	<0.001	0.71 (0.62-0.81)	<0.001
Genetics (rs, Gene,Chr, Allele1/2)	**Frequency (A1)**				
Candidate genes from literature					
ApoE ε 4 allele, chr 19	0.28	1.01 (0.95-1.08)	0.71	1.10 (1.03;1.19)	0.007
rs6997892, *WRN*, chr 8 (G/A)	0.88	0.97 (0.91-1.05)	0.48	0.92 (0.86-1.00)	0.04
rs2684766, *IGF1R*, chr 15 (T/C)	0.97	1.03 (0.88;1.19)	0.75	1.19 (1.00-1.40)	0.05
rs11630259, *IGF1R*, chr 15 (T/C)	0.73	1.04 (0.99;1.10)	0.15	1.09 (1.02;1.17)	0.02
GWA continuous mortality selection					
rs10817931, *TRIM32*, chr 9(A/C)	0.38	1.02 (0.97-1.07)	0.54	1.07 (1.01-1.13)	0.01
rs1421783, *MAT2B*, chr 5(C/G)	0.93	0.94 (0.85;1.04)	0.24	0.89 (0.80;1.00)	0.05
Socio-economics					
Social class (min = 1, max = 4 (SD))	2.59 (1.19)	0.87 (0.84;0.90)	<0.001	0.95 (0.91;0.99)	0.03
Living Situation					
independent	4941 (82.71)	reference		reference	
service flat	610 (10.21)	2.88 (2.61-3.18)	<0.001	1.04 (0.93-1.16)	0.05
home for elderly	423 (7.08)	9.06 (8.12-10.12)		1.26 (1.03-1.47)	
Lifestyle					
Smoking					
Never	2101 (35.17)	reference		reference	
Former	2491 (41.70)	0.94 (0.87-1.02)	0.53	1.07 (0.96-1.19)	<0.001
Current	1382 (23.13)	1.12 (1.02-1.23)		1.45 (1.27-1.66)	
Packyears (SD)	16.58 (23.15)	1.12 (1.08-1.16)	<0.001	1.07 (1.03-1.12)	<0.001
Nutrition					
Energy intake (kJ) (SD)	8280.21 (2133)	1.05 (0.99-1.11)	0.11	1.08 (1.02-1.13)	0.006
Physiology					
Diastolic blood pressure (mmHg) (SD)	73.71 (11.50)	0.99 (0.95;1.02)	0.49	1.05 (1.00-1.11)	0.04
Systolic blood pressure (mmHg) (SD)	139.37 (22.30)	1.37 (1.32;1.41)	<0.001	1.06 (1.00-1.13)	0.03
Body mass index (kg/m^2) (SD)	26.30 (3.71)	0.93 (0.89; 0.97)	0.05	0.86 (0.80;0.92)	<0.001
Body mass index squared (kg/m^2)^2 (SD)	705.33 (205.78)	1.04 (1.02; 1.06)		1.03 (1.01;1.05)	
Waist circumference (cm) (SD)	90.57 (11.17)	1.21 (1.16; 1.25)	<0.001	1.10 (1.04;1.17)	0.002

continued

Table 1. continued

Variable	Baseline mean (SD)/n (%)	Univariate RR (95%-CI)	p	Final Model RR (95%-CI)	p
Physiology (cont.)					
Erythrocyte Sedimentation (mm/h) (SD)	13.53 (11.89)	1.32 (1.28; 1.37)	<0.001	1.08 (1.02;1.14)	0.006
Leucocytes (10*9/l) (SD)	6.70 (1.92)	1.18 (1.15; 1.21)	<0.001	1.11 (1.07;1.15)	<0.001
Creatinine (μmol/l) (SD)	83.18 (20.52)	1.20 (1.18;1.22)	<0.001	1.06 (1.01-1.12)	0.03
C-reactive protein (SD)	3.36 (6.61)	1.21 (1.19;1.23)	<0.001	1.07 (1.03-1.10)	<0.001
Total cholesterol (mmol/l) (SD)	6.60 (1.22)	0.81 (0.78-0.84)	<0.001	0.92 (0.89-0.96)	<0.001
Bone mineral density of femoral neck (SD)	0.83 (0.14)	0.77 (0.71; 0.83)	<0.001	0.93 (0.88;0.99)	0.01
Aortic calcification (SD)	1.80 (1.49)	1660 (1.55-1.76)	<0.001	1.08 (1.01-1.16)	0.03
Diseases					
Diabetes mellitus (Yes vs. No)	618 (10.35)	2.11 (1.92;2.33)	<0.001	1.39 (1.25;1.55)	<0.001
Left ventricular hyperthrophy (Yes vs. No)	258 (4.32)	2.35 (2.04-2.70)	<0.001	1.33 (1.13;1.55)	<0.001
Atrial fibrilation (Yes vs. No)	318 (5.32)	3.28 (2.89;3.73)	<0.001	1.32 (1.15;1.51)	<0.001
Peripheral artery disease (Yes vs. No)	1133 (18.97)	2.63 (2.42;2.86)	<0.001	1.16 (1.03;1.30)	0.01
Myocardial infarction (Yes vs. No)	754 (12.62)	2.06 (1.87; 2.26)	<0.001	1.39 (1.25;1.55)	<0.001
Parkinson Disease (Yes vs. No)	64 (1.07)	4.15 (3.26;5.28)	<0.001	1.54 (1.16;2.05)	0.003
Prevalent cancer					
time 0y - 5y (Yes vs. No)	282 (4.72)	2.58 (2.05-3.24)	<0.001	2.03 (1.60-2.58)	<0.001
time > 5y (Yes vs No)	200 (3.87)	1.44 (1.18-1.76)		1.08 (0.88-1.30)	
Minimental State Examination (SD)	27.26 (2.84)	0.59 (0.58-0.61)	<0.001	0.86 (0.82-0.90)	<0.001
General Health					
Serious illness in the last 5y? (Yes vs. No)	621 (10.40)	1.48 (1.33;1.66)	<0.001	1.13 (1.00;1.28)	0.05
Unintentional weight loss? (Yes vs. No)	675 (11.30)	2.12 (1.92;2.33)	<0.001	1.22 (1.09;1.36)	<0.001
How is your general health compared to members of your age group?					
better	3083 (51.61)	reference		reference	
same	2299 (38.48)	0.97 (0.90-1.05)	<0.001	1.06 (0.97-1.15)	<0.001
worse	592 (9.91)	1.59 (1.42-1.77)		1.32 (1.14-1.53)	
Prevalent memory complaints?					
time 0y - 5y					
no memory complaints	5559 (93.06)	reference		reference	
mild memory complaints	370 (6.19)	4.86 (4.05-5.82)		1.15 (0.94-1.40)	
severe memory complaints	45 (0.75)	10.36 (7.31-14.67)		1.02 (0.64-1.63)	
time > 5y			<0.001		0.003
no memory complaints	4928 (95.41)	reference		reference	
mild memory complaints	207 (4.01)	2.61 (2.19-3.1)		1.07 (0.90-1.27)	
severe memory complaints	15 (0.29)	17.04 (10.25-28.33)		3.34 (1.82-6.11)	

Footnote Table 1:

* RR indicates relative risk; p, p-value; CI, confidence interval; SD, standard deviation, n – population; CVA, cardiovascular accident; Chr., chromosome. This table shows all variables significant in the final model. Variables included in the full model but not included in this table are: socio-economics: education, social class, occupation, insurance, living circumstance, death of spouse, lifestyle: alcohol in g/day, fruit consumption in g/day, vegetable consumption in g/day, physiology: waist circumference, high density lipoprotein cholesterol, bone mineral density of lumbar spine, bone mineral density of femoral neck, general health: specialist visit within the last month, number of specialist visits in the last year, general practitioner visit within the last month, number of general practitioner visits in the last year, hospitalization within the last year, falls in the previous month, activities of daily living, disease: gout, vertebral fracture, cardiovascular accident, transient ischemic attack, hip fracture, coronary operation.

Table 2. C-index for different combinations of risk factors at different time-points during 15-year follow-up

Group	C-Index (95%-CI)				
	0 - 1 year	0 - 3 year	0 - 5 year	0 - 10 year	0 - 15 year
Age & Gender	0.80(0.78-0.82)	0.80(0.78-0.81)	0.79(0.77-0.8)	0.77(0.76-0.78)	0.76(0.75-0.77)
Genetics	0.55(0.53-0.58)	0.55(0.53-0.58)	0.55(0.54-0.57)	0.56(0.54-0.57)	0.56(0.55-0.57)
Socio-economics	0.79(0.77-0.81)	0.78(0.76-0.8)	0.76(0.75-0.78)	0.73(0.72-0.74)	0.72(0.71-0.73)
Life Style	0.64(0.62-0.66)	0.63(0.62-0.65)	0.62(0.61-0.64)	0.60(0.59-0.61)	0.59(0.58-0.6)
General Health	0.79(0.76-0.82)	0.77(0.75-0.8)	0.75(0.73-0.77)	0.71(0.7-0.72)	0.68(0.67-0.69)
Disease	0.78(0.74-0.82)	0.76(0.73-0.79)	0.75(0.72-0.77)	0.71(0.7-0.72)	0.69(0.68-0.7)
Physiology	0.79(0.75-0.82)	0.77(0.74-0.8)	0.76(0.73-0.78)	0.73(0.72-0.75)	0.72(0.71-0.73)
Age & Gender + Socio-economics + Life Style	0.82(0.79-0.84)	0.81(0.79-0.83)	0.80(0.79-0.82)	0.78(0.78-0.79)	0.77(0.77-0.78)
Age & Gender + General Health + Disease + Physiology	0.85(0.82-0.87)	0.84(0.82-0.86)	0.83(0.81-0.84)	0.81(0.8-0.82)	0.79(0.78-0.8)
Full Model: Age & Gender + Socio-economics + Life Style + General Health + Disease + Physiology + Genetics	0.85(0.83-0.88)	0.84(0.82-0.86)	0.83(0.82-0.85)	0.81(0.81-0.82)	0.80(0.79-0.81)

Footnote Table 2: The C-indices in this table are the area under the curve of the graphs in the figures for five different time intervals (1, 3, 5, 10, and 15 years). A C-index of 0.50 indicates a prediction of mortality that is no better than chance, whereas a C-index of 1.0 reflects perfect predictive quality.

Supplementary Material

This appendix has been provided by the authors to give readers additional information about their work.

Table 1 Descriptive statistics of 8 cohorts participating in the analysis of time to death*

Study	N	N deceased	Mean age at Baseline (±SD)	Mean age at death (±SD)	Sex, % female	Mean follow-up time in years (±SD)
Cardiovascular Health Study (CHS)	3267	1718	72.3 (5.4)	83.4 (6.3)	61%	12.3 (4.2)
Framingham Heart Study (FHS)	3136	654	70.0 (10.2)	83.0 (9.3)	56%	6.0 (2.4)
Atherosclerosis Risk Communities Study (ARIC)	4511	1108	59.4 (2.9)	71.3 (5.4)	50%	15.7 (3.7)
Age, Gene/Environment Susceptibility -Reykjavik Study (AGES)	3219	558	76.4 (5.5)	79.3 (5.9)	58%	5.2(1.3)
Invecchiare nel Chianti (InCHIANTI)	902	183	72.5 (7.7)	85.4 (7.9)	56%	5.9 (0.9)
Baltimore Longitudinal Study of Ageing (BLSA)	620	183	62.0 (8.8)	86.8 (8.0)	41%	15.7 (8.2)
The Health, Aging and Body Composition (HABC)	1661	460	73.8 (2.8)	80.4 (3.7)	47%	8.2 (2.3)
Study of Health in Pomerania (SHIP)	1717	406	66.4 (7.2)	76.9 (7.2)	47%	9.2 (2.4)
TOTAL	19033	5270	68.8 (8.8)	79.8 (8.2)	55%	10.0 (5.3)

*The methodology associated with the genome-wide association study used to derive the indicator SNPs is published as Walter S, Atzmon G, Demerath EW, A genome-wide association study of aging. Neurobiol Aging. 2011 Nov;32(11):2109.e15-28. Epub 2011 Jul 22.

Table 2 Information on genotyping, quality control and imputation by cohort*

Study	RS	AGES	CHS	FHS
Array type	Version 3 Illumina Infinium II HumanHap550 SNP chip array	Illumina 370CNV	Illumina 370CNV	Affymetrix 500K and MIPS 50K combined
Genotyping center	Erasmus Medical Center	NIA Laboratory of Neurogenetics	Cedars-Sinai Medical Center	Affymetrix Core Laboratory
Genotype calling	Illumina Beadstudio	Illumina BeadStudio	Illumina BeadStudio	BRLMM
Exclusion on SNPs used for imputation	Call rate <90%, No MAF/HWE filter	Call rate <97%, HWE $p<1^{-6}$, MAF <1%, Mishap $p<1^{-9}$, A/T and G/C SNPs, Mismatches between Illumina, dbSNP and/or HapMap position	Call rate≤97%, HWE $p<10^{-5}$, >2 duplicate errors or Mendelian inconsistencies (for reference CEPH trios) heterozygote frequency=0, not in HapMap	Call rate <97%, HWE $p<1^{-6}$, Mishap $p<1^{-9}$, Mendelian errors >100
Exclusion on a per sample basis	Call rate <97.5% sex mismatch, excess autosomal heterozygosity >0.336, outliers identified by the IBS clustering analysis	Sex mismatch, Sample failure, Genotype mismatch with reference panel	Call rate <95%, sex mismatch, sample failure	Call rate <97%, subject heterozygosity >5 SD away from the mean, large Mendelian error rate
Imputation	Mach 1.0	MACH (version 1.00.16	BIMBAM10 v0.99	MACH (version 1.00.15)
Imputation Backbone (NCBI build)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)	HapMap release 22 CEU (build 36)
Data handling and statistical tests	PLINK, ProbABLE, Mach2QTL,	PLINK and R	PLINK, R	R packages kinship, GEE, COXPH

Table 2 Information on genotyping, quality control and imputation by cohort (ctd.)

Study	ARIC	HABC	ICH	BLSA	SHIP
Array type	Affymetrix GeneChip SNP Array 6.0	Illumina Human1M-Duo	Illumina 550K	Illumina 550K	Affymetrix SNP Array 6.0
Genotyping center	Broad Institute	Center for Inherited Disease Research (NIH)	Laboratory of Neurogenetics (NIA)	Laboratory of Neurogenetics (NIA)	Affymetrix, Inc./Greifswald University
Genotype calling	Birdseed	Illumina BeadStudio	BeadStudio	BeadStudio	Birdseed V2
Exclusion on SNPs used for imputation		call rate < 97%, HWE p<10-6, MAF <1%,	call rate < 99%, HWE p<10-4, MAF <1%,	call rate < 99%, HWE p<10-4, MAF <1%,	NA
Exclusion on a per sample basis	sample failure, genotypic sex mismatch, and first-degree relative,	sample failure, genotypic sex mismatch, and first-degree relative in samples set	Call rate <97%, heterozygosity < 0.3, sex mismatch	Non-european decent, call rate < 98.5%, sex mismatch, duplication	Call rate <92%, IBS duplicates, sex mismatch
Imputation	MACH v1.0.16	MACH (version 1.0.16)	MACH (version 1.0.16)	MACH (version 1.0.16)	IMPUTE v0.5.0
Imputation Backbone (NCBI build)	Hapmap CEU release 22 build 36	Hapmap CEU release 22 build 36	Hapmap CEU release 22 build 36	Hapmap CEU release 22 build 36	HapMap release 22 CEU (build 36)
Data handling and statistical tests	ProbABEL	R	ProbABEL, R, Perl	ProbABEL, R, Perl	ProbABEL, InforSense, Caché

*The methodology associated with the genome-wide association study used to derive the indicator SNPs is published as Walter S, Atzmon G, Demerath EW, A genome-wide association study of aging. Neurobiol Aging. 2011 Nov;32(11):2109.e15-28. Epub 2011 Jul 22.

Table 3 Extended version of Table 1 contained in the print version of the paper

Table 3 - Descriptive statistics and association to 15-year mortality in 5974 participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models*

non-genetic risk factors

A	B	C		D		E	F	
	Baseline	Univariate		Block adjusted		Final Model		
						Lasso	Backward	
Variable	**mean(SD)/n (%)**	**RR (95%-CI)**	**p**	**RR (95%-CI)**	**p**		**RR (95%-CI)**	**p**
Age	69.43 (9.10)	**1.12 (1.11; 1.12)**	**<0.001**			1.09	**1.09 (1.08;1.1)**	**<0.001**
Gender (female vs male)	3547 (59.37)	**0.79 (0.73;0.85)**	**<0.001**			0.72	**0.71 (0.62;0.81)**	**<0.001**
Socio-economics								
Education								
continuous min=1, max=7 (SD)	3.35 (1.93)	**0.76 (0.73;0.80)**	**<0.001**	0.96 (0.92;1.01)	0.06	xxx	xxx	xxx
Occupation								
employed	743 (12.43)	**0.25 (0.22;0.29)**		0.89 (0.75;1.04)		xxx	0.91 (0.77;1.08)	
houseman/wife <65y	565 (9.46)	**0.18 (0.15;0.22)**		0.93 (0.75;1.16)		xxx	1.02 (0.82;1.26)	
disability pension <65y	214 (3.59)	**0.31 (0.24;0.41)**	**<0.001**	1.07 (0.81;1.41)	0.51	xxx	0.99 (0.75;1.30)	0.64
living of interest / early retirement	688 (11.52)	**0.25 (0.21;0.29)**		0.84 (0.71;1.00)		0.98	0.88 (0.74;1.05)	
once employed >65y	3564 (59.66)	**reference**		reference		ref.	reference	
never employed > 65	200 (3.34)	**1.01 (0.83;1.18)**		1.11 (0.92;1.32)		xxx	1.08 (0.92;1.33)	
Income								
x1000 Euro/month (SD)	2.12 (1.02)	**0.68 (0.61;0.75)**	**<0.001**	0.95 (0.89;1.01)	0.09	0.98	0.96 (0.90;1.02)	0.17
Social Class								
Min = 1, max = 4 (SD)	2.59 (1.19)	**0.87 (0.84;0.90)**	**<0.001**	0.97 (0.93;1.01)	0.12	0.97	**0.95 (0.91;0.99)**	**0.03**
Insurance								
private vs public	2658 (44.49)	**0.66 (0.62;0.71)**	**<0.001**	0.95 (0.88; 1.04	0.30	xxx	xxx	xxx
Children								
Number of Children (SD)	2.12 (1.61)	1.02 (0.99;1.06)	0.25	xxx	xxx	xxx	xxx	xxx
Living Situation								
independent	4941 (82.71)	**reference**	**<0.001**	**reference**	**<0.001**	ref.	**reference**	**0.05**

service flat	610 (10.21)	**2.88 (2.61;3.18)**			**1.13 (1.01;1.26)**		xxx	**1.04 (0.92;1.16)**	
home for elderly	423 (7.08)	**9.06 (8.12;10.12)**			**1.97 (1.72;2.27)**		1.12	**1.26 (1.03;1.47)**	
Living Circumstances									
alone	1915 (32.06)	**1.87 (1.74; 2.02)**		**<0.001**	xxx		xxx	xxx	
With partner	3864 (64.68)	**reference**			xxx		ref.	xxx	Al
other	195 (3.26)	**0.92 (0.74; 1.15)**			xxx		0.98	xxx	
Life event: Death of Spouse									
Yes	467 (7.86)	**1.41 (1.25; 1.60)**			1.02 (0.89;1.16)		xxx	0.98 (0.88;1.12)	
No	3643 (60.98)	**reference**		**<0.001**	reference	0.89	ref.	reference	0.52
N/A	1864 (31.21)	**1.06 (0.98; 1.14)**			0.97 (0.89;1.05)		1.02	1.08 (0.99;1.18)	

Percentage missing information: age (0%), gender (0%), education (2.8%), occupation(2.7%), income (17.1%), social class (7.6%), health insurance (1.4%), children (3.6%), living situation (1.2%), living alone (5.0%), death of spouse (2.9%).

Table 3 - Descriptive statistics and association to 15-year mortality in 5974 participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models* (ctd.)

non-genetic risk factors

A	B	C		D		E	F	
	Baseline	**Univariate**		**Block adjusted**		**Final Model**		
						Lasso	**Backward**	
Lifestyle								
Alcohol								
yes vs. no	4554 (76.23)	**0.66 (0.58;0.74)**	**<0.001**	**0.77 (0.68;0.86)**	**<0.001**	0.97	0.94 (0.84;1.06)	0.31
g/day (SD)	9.92 (15.18)	**0.96 (0.92, 1.01)**	0.12	1.01 (0.96;1.07)	0.67	1.02	1.02 (0.97;1.08)	0.38
Smoking								
Never	2101 (35.17)	reference		**reference**		ref.	**reference**	
Former	2491 (41.70)	0.94 (0.87;1.02)	0.53	**1.08 (0.97;1.20)**	**<0.001**	xxx	**1.07 (0.96;1.19)**	**<0.001**
Current	1382 (23.13)	1.12 (1.02; 1.23)		**1.58 (1.41;1.78)**		1.33	**1.45 (1.27;1.66)**	
Packyears (SD)	16.58 (23.15)	**1.12 (1.08; 1.16)**	**<0.001**	**1.13 (1.09;1.17)**	**<0.001**	1.08	**1.07 (1.03;1.12)**	**<0.001**
Bike								
Do you ride bike? (no vs. yes)	2437 (40.79)	**2.76 (2.57;2.96)**	**<0.001**	**1.53 (1.28;1.53)**	**<0.001**	1.09	1.10 (0.98;1.23)	0.09
Nutrition								
Energy intake (kJ) (SD)	8280.21 (2132.93)	1.05 (0.99;1.11)	0.11	**1.10 (1.05;1.15)**	**0.01**	1.05	**1.08 (1.02;1.13)**	**0.006**
Vegetables (g/day) (SD)	345.61 (139.30)	**0.89 (0.80; 0.99)**	**0.03**	0.97 (0.92;1.02)	0.22	0.98	0.97 (0.91;1.02)	0.21
Fruit (g/day) (SD)	228.39 (132.96)	**0.93 (0.86; 1.00)**	**0.04**	0.99 (0.94;1.03)	0.56	xxx	0.98 (0.93;1.03)	0.41

Percentage missing information: alcohol (22.9%), alcohol in g/day (22.9%), smoking (2.8%), packyears (5%), bike (5.6%), energy intake (26.7%), vegetables (26.7%), fruit (26.7%).

Table 2 - Descriptive statistics and association to 15-year mortality in 5974participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models* (ctd.)

non-genetic risk factors

A	B	C		D		E	F	
	Baseline	Univariate		Block adjusted		Final Model		
						Lasso	Backward	
Physiology								
Diastolic blood pressure (mmHg) (SD)	73.71 (11.50)	0.99 (0.95;1.02)	0.49	**1.06 (1.01;1.12)**	**0.01**	1.04	**1.05 (1.00;1.10)**	**0.04**
Systolic blood pressure (mmHg) (SD)	139.37 (22.30)	**1.37 (1.32;1.41)**	**<0.001**	**1.06 (1.01;1.12)**	**0.01**	1.05	**1.06 (1.01;1.13)**	**0.03**
Anthropometry								
Weight (kg) (SD)	73.13 (12.01)	**0.86 (0.83;0.90)**	**<0.001**	**0.86 (0.78;0.94)**	**0.002**	xxx	xxx	xxx
Body mass index (kg/m^2) (SD)	26.30 (3.71)	**0.93 (0.89; 0.97)**	0.05	**0.85 (0.78;0.93)**	**0.008**	0.94	**0.86 (0.80;0.92)**	**<0.001**
Body mass index squared (kg/m^2)^2 (SD)	705.33 (205.78)	**1.04 (1.02; 1.06)**		**1.05 (1.03;1.07)**		1.02	**1.03 (1.01;1.05)**	
Waist circumference (cm) (SD)	90.57 (11.17)	1.21 (1.16; 1.25)	**<0.001**	**1.18 (1.11;1.25)**	**<0.001**	xxx	**1.10 (1.04;1.17)**	**0.002**
Hip circumference (cm) (SD)	100.11 (7.89)	0.99 (0.96;1.03)	0.72	1.05 (0.99;1.11)	0.14	xxx	xxx	xxx
Waist to hip ratio (SD)	0.91 (0.09)	**1.25 (1.21;1.30)**	**<0.001**	xxx	xxx	1.04	xxx	xxx
Blood								
Erythrocyte Sedimentation Rate(mm/h) (SD)	13.53 (11.89)	**1.32 (1.28; 1.37)**	**<0.001**	**1.10 (1.04;1.16)**	**0.001**	1.04	**1.08 (1.02;1.14)**	**0.006**
Plaques (SD)	1.63 (1.85)	**1.41 (1.28;1.56)**	**<0.001**	**1.09 (1.03;1.15)**	**0.006**	1.07	1.04 (0.98;1.11)	0.19
Leucocytes (10*9/l) (SD)	6.70 (1.92)	**1.18 (1.15; 1.21)**	**<0.001**	**1.12 (1.08;1.15)**	**<0.001**	1.09	**1.11 (1.07;1.15)**	**<0.001**
Proteins								
Albumine (g/l) (SD)	42.79 (2.76)	**0.69 (0.66;0.73)**	**<0.001**	**0.94 (0.90;0.99)**	**0.01**	0.99	0.97 (0.93;1.02)	0.29
Creatinine (µmol/l) (SD)	83.18 (20.52)	**1.20 (1.18;1.22)**	**<0.001**	**1.09 (1.04;1.14)**	**<0.001**	1.06	**1.06 (1.01;1.12)**	**0.03**
C-reactive protein (SD)	3.36 (6.61)	**1.21 (1.19;1.23)**	**<0.001**	**1.07 (1.04;1.10)**	**<0.001**	1.07	**1.07 (1.03;1.10)**	**<0.001**
Lipids								
Total cholesterol (mmol/l) (SD)	6.60 (1.22)	**0.81 (0.78;0.84)**	**<0.001**	**0.90 (0.87;0.94)**	**<0.001**	0.94	**0.92 (0.89;0.96)**	**<0.001**
Hdl-cholesterol (mmol/l) (SD)	1.34 (0.37)	**0.84 (0.81;0.87)**	**<0.001**	0.98 (0.94;1.02)	0.29	xxx	1.02 (0.98;0.96)	0.31
Miscellaneous								

Uric acid (µmol/l) (SD)	321.88 (81.69)	**1.27 (1.22;1.32)**	**<0.001**	**1.09 (1.04;1.14)**	**<0.001**	1.04	1.05 (1.00;1.11)	0.06
Insuline, 2 hours post load glucose (per SD)	65.49 (58.30)	**1.18 (1.15; 1.22)**	**<0.001**	1.04 (0.99;1.09)	0.12	Xxx	xxx	xxx
Bone mineral density of lumbar spine (SD)	1.09 (0.20)	1.03 (0.94; 1.12)	0.49	1.03 (0.97;1.10)	0.29	Xxx	1.04 (0.98;1.10)	0.18
Bone mineral density of femoral neck (SD)	0.83 (0.14)	**0.77 (0.71; 0.83)**	**<0.001**	**0.92 (0.86;0.99)**	**0.03**	0.97	**0.93 (0.88;0.99)**	**0.01**
Aortic calcification (SD)	1.82 (1.50)	**1.66 (1.55;1.76)**	**<0.001**	**1.15 (1.08;1.22)**	**<0.001**	1.06	**1.08 (1.01;1.16)**	**0.03**

Percentage missing information: diastolic blood pressure (3.1%), systolic blood pressure (3.1%), weight (3.6%), BMI (3.83%), waist circumference (8.4%), hip-circumference (8.4%) waist to hip ratio (8.5%), erythrocyte sedimentation rate (27.6%), plaques (22%), leucocytes (7.7%), albumine (26.9%), creatinine (26.5%), c-reactive protein (6.8%), total cholesterol (1.7%), Hdl-cholesterol (2.1%), uric acid (28.5%), insuline (21.4%), bone mineral density of lumbar spine (17.3%), bone mineral density of femoral neck (17.7%), aortic calcification (18.6%).

Table 2 - Descriptive statistics and association to 15-year mortality in 5974 participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models* (ctd.)

non-genetic risk factors

A	B	C		D		E	F	
	Baseline	Univariate		Block adjusted		Final Model		
						Lasso	Backward	
Diseases and Disease History								
Diabetes mellitus (Yes vs. No)	618 (10.35)	**2.11 (1.92;2.33)**	**<0.001**	**1.56 (1.41;1.74)**	**<0.001**	1.37	**1.39 (1.25;1.55)**	**<0.001**
Left ventricular hyperthrophy (Yes vs. No)	258 (4.32)	**2.35 (2.04;2.70)**	**<0.001**	**1.47 (1.27;1.70)**	**<0.001**	1.31	**1.33 (1.13;1.55)**	**<0.001**
Atrial fibrilation (Yes vs. No)	318 (5.32)	**3.28 (2.89;3.73)**	**<0.001**	**1.47 (1.29;1.68)**	**<0.001**	1.30	**1.32 (1.15;1.51)**	**<0.001**
Hypertension (Yes vs. No)	2055 (34.40)	**1.57 (1.46;1.69)**	**<0.001**	**1.18 (1.10;1.28)**	**<0.001**	1.05	1.08 (0.98;1.19)	0.10
Hip fracture (Yes vs. No)	62 (1.04)	**2.11 (1.60;2.77)**	**<0.001**	1.11 (0.83;1.49)	0.47	xxx	0.86 (0.64;1.17)	0.35
Peripheral artery disease (Yes vs. No)	1133 (18.97)	**2.63 (2.42;2.86)**	**<0.001**	**1.40 (1.27;1.54)**	**<0.001**	1.19	**1.16 (1.03;1.30)**	**0.01**
Myocardial infarction (Yes vs. No)	754 (12.62)	**2.06 (1.87; 2.26)**	**<0.001**	**1.40 (1.26;1.56)**	**<0.001**	1.30	**1.39 (1.25;1.55)**	**<0.001**
Heart failure (Yes vs. No)	194 (3.25)	**3.38 (2.89;3.96)**	**<0.001**	**1.31 (1.10;1.54)**	**<0.001**	1.15	1.17 (0.98;1.39)	0.09
Dementia (Yes vs. No)	233 (3.91)	**7.87 (6.85;9.04)**	**<0.001**	**1.24 (1.02;1.52)**	**0.03**	1.02	xxx	xxx
Gout (Yes vs. No)	42 (0.70)	1.19 (0.79;1.78)	0.3991	0.85 (0.58;1.24)	0.40	0.98	0.80 (0.54;1.20)	0.29
Parkinson Disease	64 (1.07)	**4.15 (3.26;5.28)**	**<0.001**	**1.92 (1.47;2.53)**	**<0.001**	1.52	**1.54 (1.16;2.05)**	**0.003**

(Yes vs. No)								
Cerebrovascular accident	167 (2.80)	**3.06 (2.61;3.65)**	**<0.001**	**1.25 (1.05;1.49)**	**0.01**	xxx	1.07 (0.89;1.30)	0.47
(Yes vs. No)								
Prevalent cancer								
Time 0y - 5y (Yes vs. No)	282 (4.72)	**2.58 (2.05;3.24)**	**<0.001**	**2.17 (1.72;2.73)**	**<0.001**	1.25	**2.03 (1.60;2.58)**	**<0.001**
Time > 5y (Yes vs No)	200 (3.87)	**1.44 (1.18; 1.76)**		1.18 (0.97;1.42)		**	1.08 (0.88;1.30)	
Transient ischemic attack	199 (3.33)	**1.64 (1.39;1.94)**	**<0.001**	1.11 (0.93;1.32)	0.23	xxx	xxx	xxx
(Yes vs. No)								
Minimental State examintation (SD)	27.26 (2.84)	**0.59 (0.58;0.61)**	**<0.001**	**0.82 (0.78;0.86)**	**<0.001**	0.86	**0.86 (0.82;0.90)**	**<0.001**
Coronary Operation	176 (2.95)	**1.53 (1.26; 1.86)**	**<0.001**	1.18 (0.97;1.43)	0.10	xxx	1.10 (0.90;1.34)	0.36
(Yes vs. No)								

Percentage missing information: diabetes mellitus (0.6%), left ventricular hyperthrophy (0.1%), atrial fibrilation (5.3%) , hypertension (2.6), hip fracture (4.9%), peripheral artery disease (10.8%), myocardial infarction (7.5%), heart failure (0%), dementia (0.7%), gout (3.8%), parkinson disease (5.9%), cerbrovascular accident (0%), pervalent cancer (0%), transient ischemic attack (5%), MMSE (2.9%), coronary operation (6%).

Table 3 - Descriptive statistics and association to 15-year mortality in 5974 participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models* (ctd.)

non-genetic risk factors

A	B	C		D		E	F	
	Baseline	**Univariate**		**Block adjusted**		**Final Model**		
						Lasso	**Backward**	
General Health								
Activities of Daily Living (SD)	0.44 (0.63)	**1.77 (1.72; 1.83)**	**<0.001**	**1.07 (1.00;1.14)**	**0.04**	1.04	1.05 (0.98;1.12)	0.17
Instrumental Activities of Daily Living (SD)	4.44 (5.78)	**1.98 (1.90; 2.05)**	**<0.001**	**1.17 (1.10;1.25)**	**<0.001**	1.08	1.04 (0.97;1.11)	0.33
Shortness of breath?	2119 (35.47)	**1.35 (1.24; 1.47)**	**<0.001**	1.08 (0.97;1.21)	0.16	xxx	xxx	xxx
(Yes vs. No)								
GP visit last month?	2242 (37.53)	**1.19 (1.10; 1.28)**	**<0.001**	0.97 (0.89;1.07)	0.57	xxx	xxx	xxx
(Yes vs. No)								
Number of GP visits last year? (SD)	1.33 (2.73)	**1.09 (1.06; 1.12)**	**<0.001**	**1.05 (1.01;1.09)**	**0.03**	1.02	1.03 (1.00;1.06)	0.09
Specialist visit last month?	1273 (21.32)	**1.13 (1.03;1.23)**	**0.01**	xxx	xxx	xxx	xxx	xxx
(Yes vs. No)								
Number of specialist visits last year? (SD)	0.70 (2.32)	**1.04 (1.01; 1.08)**	**0.01**	1.03 (0.99;1.06)	0.14	1.02	1.03 (0.99;1.07)	0.12
Serious illness in the last 5y?	621 (10.40)	**1.48 (1.33;1.66)**	**<0.001**	**1.21 (1.08;1.35)**	**<0.001**	1.10	**1.13 (1.00;1.28)**	**0.05**
(Yes vs. No)								
Hospitalization last year?	1052 (17.60)	**1.20 (1.10;1.31)**	**<0.001**	xxx	xxx	xxx	xxx	xxx
(Yes vs. No)								
Unintentional weight loss?	675 (11.30)	**2.12 (1.92;2.33)**	**<0.001**	**1.41 (1.28;1.57)**	**<0.001**	1.22	**1.22 (1.09;1.36)**	**<0.001**
(Yes vs. No)								
Fall in the previous month?	984 (16.47)	**1.52 (1.39;1.66)**	**<0.001**	xxx	xxx	xxx	xxx	xxx
(Yes vs. No)								
How is your general health compared to members of your age group?								
better	3083 (51.61)	reference	**<0.001**	**reference**	**<0.001**	ref.	reference	**<0.001**
same	2299 (38.48)	0.97 (0.90; 1.05)		**1.09 (1.01;1.18)**		1.02	1.06 (0.97;1.15)	

worse	592 (9.91)	**1.59 (1.42;1.77)**		**1.38 (1.21;1.58)**		1.28	**1.32 (1.14;1.53)**	
Prevalent memory complaints?								
time 0y - 5y								
no memory complaints	5559 (93.06)	**reference**		**reference**		ref.	reference	
mild memory complaints	370 (6.19)	**4.86 (4.05; 5.82)**		**1.39 (1.15;1.68)**		1.08	1.15 (0.94;1.40)	
severe memory complaints	45 (0.75)	**10.36 (7.31;14.67)**		**1.53 (1.05;2.23)**		1.10	1.02 (0.64;1.63)	
time > 5y			**<0.001**		**<0.001**			**0.003**
no memory complaints	4928 (95.41)	**reference**		**reference**				
mild memory complaints	207 (4.01)	**2.61 (2.19; 3.1)**		**1.24 (1.04;1.48)**		**	1.07 (0.90;1.27)	
severe memory complaints	15 (0.29)	**17.04 (10.25; 28.33)**		**5.14 (3.03;8.70)**		**	**3.34 (1.82;6.11)**	

Percentage missing information: Activities of Daily Living (1.7%), Instrumental Activities of Daily Living (4.8%), shortness of breath (27.3%), GP visit last month (2.8%), number of GP visits last year (3.7%), specialist visit last month (2.9%), number of specialist visits last year (3.5%), serious illness (2.9%), hospitalization (2.9%), unintentional weight loss (3.1%), fall in previous month (1.9%), comparative health (4.1%), memory complaints (3.3%).

Table 3 - Descriptive statistics and association to 15-year mortality in 5974 participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models* (ctd.)

Genetic determinants

A		B	C		D		E	F	
		Baseline	Univariate		Block adjusted		Final Model		
							Lasso	Backward	
Variable		**Frequency (Allele1)**	**RR (95%-CI)**	**p**	**RR (95%-CI)**	**p**		**RR (95%-CI)**	**p**
Candidate genes from literature									
ApoE ε 4 allele	chr 19	0.28	1.01 (0.95;1.08)	0.708	**1.105 (1.029;1.186)**	**0.006**		**1.104 (1.027;1.186)**	**0.007**
Genetics (RS, Gene,Chromosome, Allele1/Allele2)									
rs17375901	MTHFR, chr 1 (C/T)	0.94	0.94 (0.85;1.04)	0.23	xxx	xxx	xxx	xxx	xxx
rs4234259	MLH1, chr 3 (A/G)	0.54	0.96 (0.91;1.01)	0.11	xxx	xxx	xxx	xxx	xxx
rs2075799	HSPA1L, chr 6 (C/T)	0.94	0.97 (0.87;1.07)	0.53	0.951 (0.857;1.055)	0.3509	xxx	0.938 (0.843;1.045)	0.2486
rs10455777	SOD2, chr 6 (C/G)	0.88	1.04 (0.96;1.13)	0.31	xxx	xxx	xxx	xxx	xxx
rs2069827	IL6, chr 7 (G/T)	0.94	0.91 (0.81;1.01)	0.08	0.963 (0.863;1.076)	0.513	xxx	xxx	xxx
rs10950917	IL6, chr 7 (G/A)	0.64	1.00 (0.95;1.05)	0.95	xxx	xxx	xxx	xxx	xxx
rs705380	PON1, chr 7 (C/G)	0.96	1.03 (0.90;1.17)	0.70	xxx	xxx	xxx	xxx	xxx
rs17166818	PON1, chr 7 (C/A)	0.99	1.10 (0.89;1.36)	0.40	1.202 (0.967;1.493)	0.097	1.09	1.186 (0.945;1.488)	0.1393
rs13223537	PON1, chr 7 (T/C)	0.74	0.99 (0.93;1.05)	0.69	xxx	xxx	0.99	xxx	xxx
rs11998012	WRN, chr 8 (A/C)	0.75	1.03 (0.98;1.09)	0.26	1.033 (0.975;1.096)	0.2634	xxx	1.041 (0.98;1.105)	0.1852
rs11782945	WRN, chr 8 (A/G)	0.58	1.03 (0.98;1.09)	0.21	xxx	xxx	xxx	xxx	xxx
rs6997892	WRN, chr 8 (G/A)	0.88	0.97 (0.91;1.05)	0.48	0.944 (0.877;1.016)	0.1288	0.97	**0.923 (0.856;0.995)**	**0.0386**
rs7928368	CAT, chr 11 (A/G)	0.09	1.09 (1.00;1.19)	0.04	0.817 (0.586;1.139)	0.2339	xxx	0.896 (0.64;1.255)	0.5249
rs10836219	CAT, chr 11 (T/A)	0.95	0.94 (0.84;1.05)	0.30	0.745 (0.515;1.078)	0.1193	xxx	0.818 (0.562;1.191)	0.2968
rs6484720	CAT, chr 11 (G/T)	0.92	0.96 (0.88;1.04)	0.30	1.352 (0.965;1.894)	0.0788	xxx	1.165 (0.826;1.641)	0.382
rs7944397	CAT, chr 11 (G/A)	0.13	1.07 (1.00;1.16)	0.05	1.321 (0.964;1.81)	0.0826	xxx	1.114 (0.81;1.531)	0.505
rs8001253	KL, chr 13 (T/C)	0.98	0.89 (0.72;1.09)	0.25	0.629 (0.4;0.989)	0.0447	xxx	0.73 (0.458;1.162)	0.1851
rs4943016	KL, chr 13 (T/G)	0.98	1.01 (0.86;1.19)	0.90	1.371 (0.958;1.961)	0.0838	xxx	1.231 (0.858;1.767)	0.2584

rs437445	KL, chr 13 (A/C)	0.77	1.00 (0.94;1.06)	0.99	xxx	xxx	xxx	xxx	xxx	xxx	xxx
rs367243	KL, chr 13 (C/T)	0.79	0.99 (0.93;1.05)	0.77	1.037 (0.965;1.113)	0.316	xxx	xxx	xxx	xxx	xxx
rs211247	KL, chr 13 (G/C)	0.79	0.99 (0.93;1.05)	0.68	xxx	xxx	xxx	xxx	xxx	xxx	xxx
rs495392	KL, chr 13 (C/A)	0.74	1.00 (0.94;1.07)	0.93	0.955 (0.89;1.025)	0.2078	xxx	0.974 (0.915;1.037)	0.4255	xxx	xxx
rs11632057	IGF1R, chr 15 (G/T)	0.95	1.02 (0.90;1.15)	0.80	1.048 (0.923;1.189)	0.4662	1.03	1.136 (0.996;1.295)	0.0565	xxx	xxx
rs2684766	IGF1R, chr 15 (T/C)	0.97	1.03 (0.88;1.19)	0.75	1.112 (0.945;1.31)	0.1986	1.02	**1.185 (1.003;1.4)**	**0.0459**	xxx	xxx
rs2684777	IGF1R, chr 15 (T/C)	0.78	0.94 (0.88;0.99)	0.03	0.988 (0.871;1.121)	0.8618	xxx	xxx	xxx	xxx	xxx
rs1879612	IGF1R, chr 15 (T/C)	0.63	0.96 (0.91;1.01)	0.10	0.935 (0.853;1.024)	0.1524	xxx	0.939 (0.854;1.032)	0.1965	xxx	xxx
rs8030777	IGF1R, chr 15 (C/T)	0.73	0.95 (0.90;1.01)	0.09	1.075 (0.964;1.198)	0.1915	xxx	1.059 (0.946;1.185)	0.3167	xxx	xxx
rs2684780	IGF1R, chr 15 (G/C)	0.81	0.92 (0.86;0.98)	0.01	0.947 (0.821;1.093)	0.4624	0.99	0.947 (0.86;1.042)	0.2701	xxx	xxx
rs2715442	IGF1R, chr 15 (A/G)	0.68	0.94 (0.89;0.99)	0.02	xxx	xxx	0.98	xxx	xxx	xxx	xxx
rs11630259	IGF1R, chr 15 (T/C)	0.73	1.04 (0.99;1.10)	0.148	**1.071 (1.001;1.145)**	**0.0452**	1.03	**1.088 (1.015;1.166)**	**0.0164**	xxx	xxx
rs1879613	IGF1R, chr 15 (G/A)	0.81	0.92 (0.86;0.98)	0.007	xxx	xxx	xxx	xxx	xxx	xxx	xxx
rs883149	IGF1R, chr 15 (A/T)	0.21	1.00 (0.94;1.07)	0.97	xxx	xxx	xxx	xxx	xxx	xxx	xxx
rs951715	IGF1R, chr 15 (G/A)	0.35	0.96 (0.91;1.02)	0.166	1.006 (0.891;1.135)	0.9181	xxx	0.949 (0.857;1.051)	0.3168	xxx	xxx
rs7169544	IGF1R, chr 15 (A/G)	0.42	0.98 (0.93;1.03)	0.37	0.977 (0.888;1.075)	0.6413	xxx	xxx	xxx	xxx	xxx
rs2684808	IGF1R, chr 15 (G/C)	0.32	0.97 (0.92;1.03)	0.332	1.052 (0.935;1.182)	0.3955	xxx	1.105 (0.982;1.243)	0.0961	xxx	xxx
rs9920651	IGF1R, chr 15 (C/T)	0.81	1.04 (0.98;1.11)	0.22	1.062 (0.978;1.154)	0.1476	xxx	1.065 (0.978;1.16)	0.1423	xxx	xxx
rs1058696	IGF1R, chr 15 (C/T)	0.93	1.00 (0.90;1.12)	0.98	1.076 (0.96;1.207)	0.205	xxx	1.059 (0.943;1.189)	0.3283	xxx	xxx
rs4293	ACE, chr 17 (G/A)	0.45	1.05 (1.00;1.11)	0.05	xxx	xxx	xxx	xxx	xxx	xxx	xxx
rs4309	ACE, chr 17 (C/T)	0.56	1.06 (1.01;1.12)	0.02	1.041 (0.937;1.155)	0.4513	xxx	1.057 (0.945;1.183)	0.3276	xxx	xxx
rs4311	ACE, chr 17 (T/C)	0.48	1.06 (1.01;1.12)	0.01	**1.15 (1.022;1.295)**	**0.0199**	1.05	1.126 (0.998;1.271)	0.0538	xxx	xxx
rs4343	ACE, chr 17 (G/A)	0.53	1.06 (1.01;1.11)	0.02	0.911 (0.775;1.07)	0.2567	xxx	0.93 (0.79;1.094)	0.3841	xxx	xxx
rs4461142	ACE, chr 17 (T/C)	0.49	1.04 (0.99;1.09)	0.14	0.969 (0.888;1.058)	0.4922	xxx	0.97 (0.889;1.059)	0.504	xxx	xxx
rs4817414	SOD1, chr 21 (G/C)	0.69	1.00 (0.95;1.05)	0.98	1.016 (0.962;1.073)	0.5569	xxx	xxx	xxx	xxx	xxx

Table 3 - Descriptive statistics and association to 15-year mortality in 5973 participants from the Rotterdam Study: unadjusted, block adjusted, and final adjusted models* (ctd.)

genetic determinants

A		B	C		D		E	F	
		Baseline	Univariate		Block adjusted		Final Model		
							Lasso	Backward	
Variable		Frequency (Allele1)	RR (95%-CI)	p	RR (95%-CI)	p		RR (95%-CI)	p
GWA continuous mortality selection									
Genetics (RS, Gene,Chromosome, Allele1/Allele2)									
rs12944527	LGALS9, chr 17(A/T)	0.57	1.057 (1.005;1.112)	0.029	1.033 (0.982;1.088)	0.2018	xxx	1.032 (0.979;1.088)	0.2279
rs7033027	ACO1, chr 9(T/C)	0.95	1.07 (0.947;1.208)	0.276	1.037 (0.917;1.173)	0.5552	xxx	xxx	xxx
rs4757638	HPS5, chr 11(A/G)	0.65	1.003 (0.95;1.058)	0.910	0.962 (0.911;1.016)	0.1703	xxx	0.968 (0.915;1.024)	0.2591
rs1881523	TRIM61, chr 4(A/G)	0.27	0.963 (0.91;1.019)	0.200	xxx	xxx	xxx	xxx	xxx
rs2892165	TFAP4, chr 16(A/T)	0.51	1.023 (0.974;1.076)	0.350	xxx	xxx	xxx	xxx	xxx
rs9997795	LARP7, chr 4(A/G)	0.36	1.022 (0.97;1.077)	0.401	1.016 (0.963;1.072)	0.5483	xxx	1.032 (0.978;1.088)	0.2499
rs2739260	CACNA1B, chr 9(A/G)	0.5	0.998 (0.948;1.051)	0.946	xxx	xxx	xxx	1.093 (0.94;1.271)	0.2439
rs17028819	CNTN3, chr 3(T/G)	0.97	1.008 (0.874;1.163)	0.911	1.1 (0.951;1.273)	0.1976	xxx	xxx	xxx
rs2533300	BRAF, chr 7(C/G)	0.1	0.992 (0.916;1.073)	0.844	1.046 (0.966;1.133)	0.2649	xxx	xxx	xxx
rs12660477	COL21A1, chr 6(A/C)	0.09	0.946 (0.857;1.044)	0.271	0.949 (0.858;1.049)	0.307	xxx	0.955 (0.861;1.059)	0.3878
rs751	LMO4, chr 1(A/G)	0.16	1.048 (0.983;1.119)	0.148	0.947 (0.887;1.012)	0.1141	xxx	0.959 (0.895;1.027)	0.2353
rs2521271	TSPAN32, chr 11(A/T)	0.9	1.006 (0.932;1.087)	0.866	xxx	xxx	xxx	xxx	xxx
rs16902508	MYC, chr 8(A/G)	0.18	0.969 (0.911;1.031)	0.327	0.974 (0.915;1.036)	0.4092	0.97	0.951 (0.891;1.015)	0.1313
rs6941028	C6orf118, chr 6(A/G)	0.85	1.028 (0.957;1.104)	0.443	1.075 (0.999;1.156)	0.051	1.02	1.046 (0.97;1.127)	0.2388
rs17171686	C7orf10, chr 7(A/G)	0.89	0.991 (0.918;1.071)	0.837	xxx	xxx	xxx	xxx	xxx
rs4850695	HECW2, chr 2(A/G)	0.76	1.048 (0.988;1.112)	0.118	1.053 (0.992;1.117)	0.0861	xxx	xxx	xxx
rs7234113	KIAA0427, chr 18(T/C)	0.17	0.98 (0.917;1.047)	0.557	xxx	xxx	xxx	xxx	xxx
rs3796834	SLC2A9, chr 4(T/C)	0.27	0.969 (0.917;1.023)	0.261	0.98 (0.927;1.035)	0.4748	0.99	0.956 (0.904;1.012)	0.1231
rs6026069	C20orf85, chr 20(T/C)	0.91	0.989 (0.904;1.082)	0.815	0.958 (0.873;1.05)	0.3618	xxx	0.966 (0.88;1.061)	0.481
rs17678034	DNER, chr 2(T/C)	0.38	1.049 (0.996;1.104)	0.067	1.039 (0.986;1.095)	0.1436	xxx	1.022 (0.969;1.078)	0.4123
rs11098163	LOC91431, chr 4(A/G)	0.83	1.003 (0.936;1.074)	0.926	0.972 (0.906;1.042)	0.4323	xxx	xxx	xxx

rs10817931	TRIM32, chr 9(A/C)	0.38	1.016 (0.965;1.069)	0.536	1.048 (0.996;1.104)	0.0674	1.04	**1.068 (1.013;1.125)**	**0.0135**
rs12518724	FGF10, chr 5(A/G)	0.16	1.036 (0.97;1.106)	0.281	1.047 (0.98;1.118)	0.1727	xxx	1.022 (0.955;1.094)	0.5136
rs3128591	COL5A1, chr 9(A/G)	0.75	1.01 (0.955;1.068)	0.705	1.051 (0.993;1.112)	0.0822	1.02	1.057 (0.997;1.121)	0.0587
rs11604904	MICAL2, chr 11(A/G)	0.21	1.036 (0.971;1.105)	0.281	1.035 (0.97;1.105)	0.2896	xxx	xxx	xxx
rs12878138	DAAM1, chr 14(T/C)	0.07	0.985 (0.896;1.082)	0.758	1.047 (0.951;1.152)	0.3446	xxx	1.05 (0.952;1.157)	0.3257
rs17688808	PCDH7, chr 4(T/C)	0.9	1.035 (0.946;1.133)	0.446	1.101 (1.004;1.206)	0.0389	1.02	1.077 (0.98;1.183)	0.1211
rs1624472	RPS24, chr 10(T/C)	0.26	0.984 (0.93;1.04)	0.570	1.069 (0.937;1.22)	0.3195	xxx	xxx	xxx
rs7167722	RAB8B, chr 15(T/C)	0.96	1.088 (0.955;1.239)	0.201	0.966 (0.892;1.046)	0.4014	xxx	1.057 (0.925;1.208)	0.4118
rs9838384	KBTBD8, chr 3(T/C)	0.11	0.987 (0.912;1.067)	0.744	xxx	xxx	xxx	xxx	xxx
rs6770087	TMEM108, chr 3(T/C)	0.38	1.007 (0.955;1.06)	0.792	xxx	xxx	xxx	xxx	xxx
rs2730265	VIPR2, chr 7(T/C)	0.68	1.025 (0.972;1.08)	0.352	1.034 (0.98;1.09)	0.2181	1.02	1.055 (0.998;1.114)	0.055
rs4675743	IDH1, chr 2(A/C)	0.92	0.979 (0.898;1.068)	0.643	xxx	xxx	xxx	xxx	xxx
rs17351982	PAX7, chr 1(T/C)	0.05	1.006 (0.879;1.153)	0.921	1.083 (0.942;1.245)	0.2582	1.05	1.133 (0.983;1.307)	0.0846
rs1421783	MAT2B, chr 5(C/G)	0.93	0.94 (0.849;1.04)	0.235	0.925 (0.836;1.024)	0.135	0.97	**0.894 (0.8;0.999)**	**0.0498**
rs10487920	CNTNAP2, chr 7(A/C)	0.17	0.998 (0.934;1.066)	0.957	xxx	xxx	1.03	xxx	xxx
rs12102024	LOC283767, chr 15(T/C)	0.7	0.989 (0.938;1.044)	0.709	xxx	xxx	xxx	xxx	xxx
rs10242191	BMPER, chr 7(A/G)	0.06	0.998 (0.896;1.11)	0.972	0.956 (0.859;1.065)	0.4216	xxx	0.969 (0.868;1.082)	0.5819
rs2526630	HDAC9, chr 7(T/C)	0.47	0.952 (0.906;1)	0.054	xxx	xxx	xxx	xxx	xxx
rs1860102	CACNA1C, chr 12(A/G)	0.05	1.097 (0.974;1.237)	0.125	1.057 (0.937;1.192)	0.3624	xxx	1.057 (0.937;1.192)	0.3624
rs9888224	CSRP3, chr 11(T/C)	0.11	1.087 (1.002;1.179)	0.044	1.056 (0.971;1.147)	0.1978	1.03	1.056 (0.971;1.147)	0.1978
rs2065558	FLRT3, chr 20(T/C)	0.4	1.02 (0.97;1.072)	0.429	1.039 (0.987;1.092)	0.1375	1.01	1.039 (0.987;1.092)	0.1375
rs12436083	EAPP, chr 14(T/C)	0.04	0.957 (0.81;1.13)	0.608	0.91 (0.769;1.078)	0.2772	xxx	0.91 (0.769;1.078)	0.2772
rs270311	LOC389435, chr 6(A/G)	0.53	0.997 (0.945;1.052)	0.925	xxx	xxx	xxx	xxx	xxx
rs4959895	FAM50B, chr 6(A/G)	0.2	0.992 (0.927;1.062)	0.836	0.973 (0.908;1.042)	0.4398	xxx	0.973 (0.908;1.042)	0.4398
rs2214060	PPP1R3A, chr 7(A/G)	0.75	0.995 (0.941;1.053)	0.884	xxx	xxx	xxx	xxx	xxx
rs16957560	CDH13, chr 16(A/G)	0.08	1.027 (0.922;1.144)	0.620	xxx	xxx	xxx	xxx	xxx
rs1855982	AK3L1, chr 1(A/G)	0.61	1.019 (0.968;1.072)	0.458	0.979 (0.93;1.03)	0.4217	xxx	0.979 (0.93;1.03)	0.4217
rs1458727	MPPED2, chr 11(T/C)	0.24	1.012 (0.955;1.072)	0.667	1.033 (0.974;1.095)	0.2771	xxx	1.033 (0.974;1.095)	0.2771
rs194867	ORC5L, chr 7(T/G)	0.43	1.023 (0.973;1.076)	0.360	xxx	xxx	1.01	xxx	xxx

Table 4 Final Model for CVD Mortality and Total Mortality Stratified by Baseline Disease Status

Variable		CVD Mortality*		No Prevalent Disease**		Any Prevalent Disease***	
		HR (95%-CI)	p	HR (95%-CI)	p	HR (95%-CI)	p
Age		1.1 (1.08;1.11)	<.0001	1.13 (1.11;1.16)	<.0001	1.08 (1.07;1.09)	<.0001
Gender (female vs male)		0.77 (0.61;0.96)	0.0203	0.61 (0.43;0.88)	0.0086	0.74 (0.63;0.86)	0.0002
Socio-economics							
Occupation							
	employed	1.08 (0.8;1.44)		1.25 (0.89;1.74)		0.86 (0.71;1.05)	
	houseman/wife <65y	1.11 (0.73;1.69)		1.35 (0.87;2.1)		0.98 (0.74;1.29)	
	disability pension <65y	1.23 (0.75;2.02)	0.33	1.36 (0.81;2.3)	0.10	0.84 (0.6;1.19)	0.14
	living of interest / early retirement	0.85 (0.58;1.23)		1.29 (0.91;1.82)		0.78 (0.62;0.97)	
	once employed >65y	reference		reference		reference	
	never employed > 65	1.34 (0.98;1.85)		1.04 (0.6;1.8)		1.05 (0.84;1.31)	
Income							
	x1000 Euro/month (SD)	0.97 (0.89;1.07)	0.53	0.94 (0.83;1.06)	0.2959	0.96 (0.89;1.03)	0.24
Social Class							
	Min = 1, max = 4 (SD)	0.97 (0.9;1.05)	0.4739	0.97 (0.88;1.08)	0.627	0.94 (0.89;0.98)	0.0059
Living Situation							
	independent	reference		reference		reference	
	service flat	1.06 (0.87;1.3)	0.08	0.98 (0.7;1.36)	0.81	1.07 (0.94;1.22)	0.05
	home for elderly	1.36 (1.01;1.85)		1.14 (0.46;2.85)		1.23 (1.01;1.5)	
Life event: Death of Spouse							
	Yes	0.9 (0.71;1.14)		0.95 (0.66;1.35)		1 (0.87;1.16)	
	No	reference	0.56	reference	0.95	reference	0.41
	N/A	1.01 (0.86;1.19)		1.04 (0.86;1.27)		1.08 (0.98;1.19)	
Lifestyle							
Alcohol							
	yes vs. no	1.02 (0.83;1.27)	0.8283	0.95 (0.74;1.22)	0.6994	0.92 (0.82;1.04)	0.1895
	g/day (SD)	0.96 (0.88;1.05)	0.3913	1.02 (0.93;1.13)	0.632	1.03 (0.97;1.09)	0.3369

Smoking							
	Never	reference		reference		reference	
	Former	1.24 (1.03;1.49)	<.0001	0.94 (0.74;1.2)	0.27	1.1 (0.97;1.23)	<.0001
	Current	1.54 (1.21;1.96)		1.41 (1.03;1.93)		1.43 (1.23;1.67)	
	Packyears (SD)	1.03 (0.97;1.11)	0.3413	1.2 (1.09;1.33)	0.0004	1.06 (1.01;1.11)	0.0249
Bike							
	Do you ride bike? (no vs. yes)	1.16 (0.97;1.39)	0.1038	1.19 (0.95;1.49)	0.14	1.09 (0.98;1.23)	0.1211
Nutrition							
	Energy intake (kJ) (SD)	1.09 (0.99;1.19)	0.0805	1.09 (0.97;1.22)	0.1652	1.06 (1;1.13)	0.0374
	Vegetables (g/day) (SD)	0.95 (0.87;1.05)	0.3165	0.96 (0.87;1.06)	0.3879	0.97 (0.91;1.04)	0.4208
	Fruit (g/day) (SD)	1.01 (0.91;1.13)	0.8039	0.94 (0.86;1.03)	0.1798	0.99 (0.93;1.05)	0.7416
Physiology							
	Diastolic blood pressure (mmHg) (SD)	1.14 (1.05;1.24)	0.0023	1.03 (0.9;1.18)	0.6799	1.03 (0.98;1.09)	0.2241
	Systolic blood pressure (mmHg) (SD)	1.08 (0.97;1.19)	0.1661	1.14 (0.95;1.38)	0.1501	1.06 (1;1.13)	0.043
Anthropometry							
	Body mass index (kg/m^2) (SD)	0.88 (0.77;1.01)	0.18	0.81 (0.69;0.95)	0.10	0.84 (0.78;0.9)	<.0001
	Body mass index squared $(kg/m^2)^2$ (SD)	1.04 (1.01;1.07)		1.08 (1.02;1.15)		1.03 (1.01;1.05)	
	Waist circumference (cm) (SD)	1.12 (0.99;1.26)	0.0654	1.09 (0.91;1.31)	0.3211	1.11 (1.03;1.2)	0.0086
Blood							
	Erythrocyte Sedimentation Rate(mm/h) (SD)	1.06 (0.97;1.16)	0.1916	1.08 (0.96;1.21)	0.1839	1.08 (1.03;1.14)	0.0049
	Plaques (SD)	1.07 (0.95;1.21)	0.2173	1.01 (0.89;1.14)	0.9213	1.07 (0.99;1.16)	0.0913
	Leucocytes (10*9/l) (SD)	1.1 (1.03;1.17)	0.0033	1.09 (1.02;1.18)	0.0173	1.1 (1.05;1.15)	<.0001
Proteins							
	Albumine (g/l) (SD)	1 (0.91;1.09)	0.9342	0.97 (0.84;1.11)	0.6398	0.99 (0.92;1.06)	0.6534
	Creatinine (µmol/l) (SD)	1.13 (1.05;1.21)	0.0011	0.94 (0.79;1.12)	0.4803	1.1 (1.05;1.16)	0.0002
	C-reactive protein (SD)	1.1 (1.04;1.15)	0.0002	1.16 (1.01;1.33)	0.0422	1.07 (1.04;1.11)	<.0001
Lipids							
	Total cholesterol (mmol/l) (SD)	1.01 (0.94;1.08)	0.8444	0.93 (0.85;1.02)	0.1186	0.92 (0.88;0.96)	0.0002

	Hdl-cholesterol (mmol/l) (SD)	1.03 (0.96;1.11)	0.455	0.94 (0.85;1.04)	0.266	1.01 (0.96;1.06)	0.6732
Miscellaneous							
	Uric acid (µmol/l) (SD)	1.06 (0.97;1.16)	0.2	1.18 (1.01;1.38)	0.0345	1.06 (1;1.13)	0.0605
	Bone mineral density of lumbar spine (SD)	1.09 (0.98;1.2)	0.1081	1.16 (1.04;1.3)	0.0085	1.02 (0.96;1.08)	0.4985
	Bone mineral density of femoral neck (SD)	0.91 (0.82;1.01)	0.0822	0.88 (0.76;1.02)	0.0818	0.95 (0.9;1.01)	0.1047
	Aortic calcification (SD)	1.17 (1.02;1.33)	0.027	1.09 (0.98;1.2)	0.1199	1.08 (1;1.18)	0.0491
Diseases and Disease History							
Diabetes mellitus	(Yes vs. No)	1.64 (1.38;1.95)	<.0001	DNA		DNA	
Left ventricular hyperthrophy	(Yes vs. No)	1.52 (1.19;1.94)	0.0008	DNA		DNA	
Atrial fibrilation	(Yes vs. No)	1.39 (1.11;1.74)	0.0039	DNA		DNA	
Hypertension	(Yes vs. No)	1.12 (0.95;1.32)	0.1833	DNA		DNA	
Hip fracture	(Yes vs. No)	0.77 (0.42;1.4)	0.3871	DNA		DNA	
Peripheral artery disease	(Yes vs. No)	1.25 (1.02;1.53)	0.0315	DNA		DNA	
Myocardial infarction	(Yes vs. No)	1.62 (1.34;1.95)	<.0001	DNA		DNA	
Heart failure	(Yes vs. No)	1.39 (1.07;1.79)	0.0127	DNA		DNA	
Gout	(Yes vs. No)	0.82 (0.45;1.52)	0.5322	DNA		DNA	
Parkinson Disease	(Yes vs. No)	1.03 (0.59;1.81)	0.9182	DNA		DNA	
Cerebrovascular accident	(Yes vs. No)	1.48 (1.09;2)	0.0121	DNA		DNA	
Prevalent cancer				DNA		DNA	
	No cancer	reference					
	Time 0y - 5y (Yes vs. No)	2.84 (1.67;4.82)	0.09	DNA		DNA	
	No cancer	Reference					
	Time > 5y (Yes vs No)	0.63 (0.43;0.93)		DNA		DNA	
Minimental State examintation (SD)		1 (0.92;1.08)	0.983	0.87 (0.75;1.01)	0.0664	0.87 (0.83;0.91)	<.0001
Coronary Operation	(Yes vs. No)	1.44 (1.07;1.96)	0.0177	DNA		DNA	
General Health							
Activities of Daily Living (SD)		0.99 (0.87;1.12)	0.8564	1.22 (1.03;1.45)	0.0201	1.02 (0.94;1.09)	0.6634
Instrumental Activities of Daily Living (SD)		1.05 (0.92;1.2)	0.4628	0.89 (0.73;1.09)	0.2406	1.12 (1.03;1.21)	0.0079
How is your general health compared to members of your age group?							

	better	reference		reference		reference	
	same	1.15 (0.99;1.34)	0.02	1.14 (0.94;1.39)	0.19	1.04 (0.94;1.14)	0.003
	worse	1.34 (1.03;1.74)		1.21 (0.82;1.79)		1.32 (1.14;1.53)	
Unintentional weight loss?	(Yes vs. No)	1.19 (0.98;1.43)	0.0789	1.36 (0.97;1.91)	0.0705	1.24 (1.1;1.4)	0.0004
Serious illness in the last 5y?	(Yes vs. No)	1.19 (0.97;1.46)	0.094	1.05 (0.73;1.51)	0.7926	1.18 (1.04;1.34)	0.0123
Number of GP visits last year? (SD)		1.04 (0.99;1.1)	0.1326	1.03 (0.95;1.11)	0.4838	1.02 (0.98;1.06)	0.2384
Number of specialist visits last year? (SD)		1 (0.92;1.08)	0.9529	1 (0.92;1.1)	0.9613	1.03 (0.99;1.08)	0.113
Prevalent memory complaints?							
time 0y - 5y							
	no memory complaints	reference		reference		reference	
	mild memory complaints	3.87 (2.71;5.52)		1.16 (0.72;1.88)	0.59	1.12 (0.97;1.3)	0.16
	severe memory complaints	0.82 (0.59;1.15)		3.22 (0.02;496.72)		1.24 (0.82;1.87)	
time > 5y			0.10				
	no memory complaints	reference					
	mild memory complaints	1.24 (0.41;3.7)					
	severe memory complaints	1.16 (0.37;3.58)					
Candidate genes from literature							
ApoE ε 4 allele	chr 19	0.98 (0.85;1.13)	0.7697	1.2 (1.02;1.4)	0.0283	1.07 (0.98;1.16)	0.1518
rs2075799	HSPA1L, chr 6 (C/T)	1.12 (0.91;1.36)	0.2855	0.93 (0.71;1.2)	0.553	0.96 (0.85;1.09)	0.5469
rs17166818	PON1, chr 7 (C/A)	1.11 (0.75;1.64)	0.5946	1.57 (0.86;2.87)	0.1453	1.09 (0.85;1.4)	0.4863
rs11998012	WRN, chr 8 (A/C)	1.05 (0.95;1.17)	0.3213	1.07 (0.93;1.22)	0.333	1.03 (0.95;1.1)	0.4994
rs6997892	WRN, chr 8 (G/A)	0.85 (0.75;0.97)	0.0165	0.91 (0.75;1.1)	0.3036	0.96 (0.88;1.05)	0.3998
rs7928368	CAT, chr 11 (A/G)	1.35 (0.72;2.53)	0.3481	0.79 (0.32;1.96)	0.6073	0.92 (0.63;1.32)	0.6425
rs10836219	CAT, chr 11 (T/A)	1.19 (0.59;2.39)	0.6251	0.71 (0.26;1.94)	0.5004	0.84 (0.55;1.28)	0.4266
rs6484720	CAT, chr 11 (G/T)	0.93 (0.48;1.78)	0.8218	1.21 (0.5;2.91)	0.6675	1.16 (0.79;1.7)	0.4429
rs7944397	CAT, chr 11 (G/A)	0.8 (0.43;1.46)	0.4617	1.13 (0.49;2.58)	0.775	1.14 (0.81;1.62)	0.4538
rs8001253	KL, chr 13 (T/C)	0.61 (0.25;1.46)	0.2663	0.38 (0.12;1.21)	0.1002	0.83 (0.49;1.41)	0.4988
rs4943016	KL, chr 13 (T/G)	1.65 (0.81;3.37)	0.1675	1.69 (0.71;4.01)	0.2318	1.16 (0.77;1.76)	0.4703
rs495392	KL, chr 13 (C/A)	0.97 (0.87;1.09)	0.6479	0.93 (0.8;1.08)	0.3377	0.99 (0.92;1.06)	0.7506
rs11632057	IGF1R, chr 15 (G/T)	1.08 (0.86;1.35)	0.5252	0.92 (0.68;1.23)	0.5612	1.19 (1.02;1.39)	0.0307

rs2684766	IGF1R, chr 15 (T/C)	0.98 (0.75;1.3)	0.9053	1.32 (0.86;2.04)	0.2	1.16 (0.96;1.41)	0.1278
rs1879612	IGF1R, chr 15 (T/C)	0.85 (0.72;1.01)	0.0573	1.14 (0.91;1.43)	0.2461	0.93 (0.83;1.03)	0.1605
rs8030777	IGF1R, chr 15 (C/T)	1.2 (0.99;1.46)	0.0643	0.94 (0.7;1.26)	0.6752	1.08 (0.95;1.22)	0.2499
rs2684780	IGF1R, chr 15 (G/C)	0.94 (0.79;1.12)	0.496	0.83 (0.63;1.1)	0.196	0.95 (0.85;1.06)	0.3465
rs11630259	IGF1R, chr 15 (T/C)	1.14 (1.01;1.29)	0.0343	1.04 (0.87;1.24)	0.6971	1.09 (1;1.18)	0.0418
rs951715	IGF1R, chr 15 (G/A)	1.02 (0.85;1.21)	0.8552	0.99 (0.78;1.25)	0.9261	0.94 (0.83;1.05)	0.2677
rs2684808	IGF1R, chr 15 (G/C)	1.05 (0.85;1.3)	0.6354	0.92 (0.7;1.21)	0.5357	1.16 (1.01;1.33)	0.0367
rs9920651	IGF1R, chr 15 (C/T)	1.04 (0.9;1.21)	0.5588	1.01 (0.81;1.26)	0.9075	1.07 (0.97;1.18)	0.1666
rs1058696	IGF1R, chr 15 (C/T)	1.01 (0.82;1.24)	0.9277	0.86 (0.66;1.13)	0.2815	1.12 (0.98;1.29)	0.0981
rs4309	ACE, chr 17 (C/T)	1.23 (1.02;1.48)	0.0321	1.1 (0.87;1.4)	0.4323	0.99 (0.87;1.13)	0.9168
rs4311	ACE, chr 17 (T/C)	1.17 (0.94;1.46)	0.1522	1.1 (0.82;1.47)	0.542	1.14 (0.99;1.31)	0.0663
rs4343	ACE, chr 17 (G/A)	0.73 (0.55;0.97)	0.0279	0.9 (0.61;1.32)	0.5893	0.97 (0.8;1.17)	0.7274
rs4461142	ACE, chr 17 (T/C)	0.96 (0.82;1.12)	0.6	0.94 (0.76;1.16)	0.5605	0.99 (0.89;1.09)	0.8206
GWA continuous mortality selection							
rs12944527	LGALS9, chr 17(A/T)	1.02 (0.92;1.12)	0.7307	1.08 (0.96;1.22)	0.2122	1.03 (0.97;1.1)	0.2922
rs4757638	HPS5, chr 11(A/G)	0.97 (0.88;1.07)	0.5202	1 (0.88;1.15)	0.9618	0.96 (0.9;1.03)	0.2752
rs9997795	LARP7, chr 4(A/G)	1.05 (0.96;1.16)	0.2887	1.12 (0.98;1.28)	0.0832	1 (0.93;1.06)	0.9005
rs17028819	CNTN3, chr 3(T/G)	1.18 (0.9;1.56)	0.2245	1.38 (0.95;1.99)	0.0912	1.05 (0.89;1.25)	0.5537
rs12660477	COL21A1, chr 6(A/C)	1.03 (0.87;1.23)	0.7167	0.97 (0.77;1.22)	0.7827	0.94 (0.84;1.06)	0.3176
rs751	LMO4, chr 1(A/G)	0.96 (0.85;1.08)	0.4535	0.92 (0.78;1.08)	0.2913	0.95 (0.88;1.03)	0.2448
rs16902508	MYC, chr 8(A/G)	1.03 (0.91;1.15)	0.6736	0.9 (0.77;1.06)	0.1977	0.96 (0.89;1.03)	0.2745
rs6941028	C6orf118, chr 6(A/G)	1.06 (0.93;1.21)	0.4128	1.15 (0.95;1.4)	0.1449	1.04 (0.95;1.13)	0.4062
rs3796834	SLC2A9, chr 4(T/C)	0.94 (0.85;1.04)	0.2184	0.98 (0.85;1.13)	0.7761	0.95 (0.89;1.01)	0.1245
rs6026069	C20orf85, chr 20(T/C)	0.97 (0.82;1.14)	0.6911	0.74 (0.58;0.93)	0.0118	1 (0.9;1.11)	0.9725
rs17678034	DNER, chr 2(T/C)	1.04 (0.95;1.15)	0.3765	1.03 (0.91;1.17)	0.6106	1.02 (0.96;1.09)	0.4376
rs10817931	TRIM32, chr 9(A/C)	1.07 (0.98;1.17)	0.1528	1.01 (0.89;1.15)	0.8239	1.08 (1.02;1.14)	0.0142
rs12518724	FGF10, chr 5(A/G)	0.91 (0.81;1.02)	0.0939	0.98 (0.82;1.16)	0.7936	1.02 (0.95;1.11)	0.551
rs3128591	COL5A1, chr 9(A/G)	1.04 (0.94;1.15)	0.437	1.06 (0.93;1.21)	0.3805	1.06 (1;1.14)	0.0582
rs12878138	DAAM1, chr 14(T/C)	0.99 (0.84;1.18)	0.9532	1.24 (0.97;1.59)	0.0891	1.01 (0.9;1.13)	0.8463
rs17688808	PCDH7, chr 4(T/C)	0.96 (0.82;1.13)	0.6593	1.15 (0.91;1.46)	0.2422	1.08 (0.97;1.21)	0.1561

rs7167722	RAB8B, chr 15(T/C)	1.05 (0.83;1.33)	0.6776	0.88 (0.64;1.19)	0.3941	1.08 (0.93;1.26)	0.3205
rs2730265	VIPR2, chr 7(T/C)	1.01 (0.91;1.11)	0.9077	0.96 (0.85;1.09)	0.5038	1.08 (1.01;1.15)	0.0168
rs17351982	PAX7, chr 1(T/C)	1.08 (0.84;1.38)	0.5538	1 (0.73;1.36)	0.9928	1.15 (0.97;1.36)	0.1117
rs1421783	MAT2B, chr 5(C/G)	0.95 (0.79;1.14)	0.579	0.86 (0.67;1.09)	0.2121	0.9 (0.79;1.02)	0.1054
rs10242191	BMPER, chr 7(A/G)	0.99 (0.81;1.2)	0.8888	0.92 (0.7;1.21)	0.5499	0.98 (0.87;1.11)	0.7725
rs1860102	CACNA1C, chr 12(A/G)	1.25 (0.99;1.59)	0.0648	1.1 (0.79;1.52)	0.5626	1.04 (0.9;1.21)	0.5769
rs9888224	CSRP3, chr 11(T/C)	1.08 (0.93;1.25)	0.31	1 (0.79;1.26)	0.9767	1.07 (0.97;1.18)	0.1615
rs2065558	FLRT3, chr 20(T/C)	1.07 (0.97;1.17)	0.1641	1.05 (0.93;1.19)	0.4148	1.01 (0.95;1.07)	0.6981
rs12436083	EAPP, chr 14(T/C)	0.81 (0.61;1.08)	0.1545	0.88 (0.58;1.33)	0.5273	0.97 (0.77;1.21)	0.7606
rs1855982	AK3L1, chr 1(A/G)	1.04 (0.95;1.14)	0.4372	0.99 (0.87;1.13)	0.8513	0.98 (0.92;1.04)	0.5396
rs1458727	MPPED2, chr 11(T/C)	1.08 (0.97;1.21)	0.1445	1.03 (0.89;1.2)	0.6647	1.03 (0.96;1.11)	0.3846

* CVD mortality: n= 5974, CVD-death = 1026
** No prevalent disease, total mortality: n=2095, death=680
*** prevalent disease, total mortality: n= 3879; death=2494

5. Discussion

The goal of this thesis is to identify risk factors for disability, disease-free survival, mortality and longevity. This general discussion provides answers to the following research questions which were formulated in the introduction and addressed in prior chapters:

1. What genetic loci are associated with longevity and time to death and disease?
2. Do body mass index, physical activity, and happiness influence time to death and time spent with disability?
3. Which set of risk factors best predicts death and how do different groups of risk factors compare in their predictive power?

In addition to answering the research questions, the methodological challenges will be described and the answers will be put in the context of individual and population ageing. The discussion will conclude with policy implications and a short description of future research directions particularly within longitudinal cohort studies.

Answers to the research questions:

1. **What genetic loci are associated with longevity and time to death and disease?**

Although we combined genetic data of up to 25 000 individuals, we were unable to conclusively identify genetic markers of ageing. When investigating longevity by comparing persons that survived to age 90 and older with persons that died before the age of 80, the strongest association was observed for rs9664222, reaching $p = 6.77 \times 10^{-7}$ after meta-analyses with the replication cohorts. The single nucleotide polymorphism lies in a region near *MINPP1* (chromosome 10), a well conserved gene involved in regulation of cellular proliferation. When turning our attention to the healthy ageing phenotype and counting person years until the onset of major disease or death, we found fourteen independent single nucleotide polymorphisms that predicted risk of death, and eight that predicted event-free survival ($p < 10^{-5}$). These single nucleotide polymorphisms are in or near genes that are highly expressed in the brain (*HECW2*, *HIP1*, *BIN2*, *GRIA1*), or involved in neural development and function (*KCNQ4*, *LMO4*, *GRIA1*, *NETO1*) and autophagy (*ATG4C*). Interestingly, while we could not unequivocally confirm popular ageing pathways, there was some evidence for networks of processes of cellular maintenance and cellular death associated with the phenotypes of time-to-event and time-to-death. We also found evidence for the association of neural plasticity in the brain with these phenotypes.

2. **Do body mass index, physical activity, and happiness influence time to death and time spent with disability?**

We showed that obesity, physical inactivity, and positive affect influence morbidity and mortality in various ways. While overweight and obesity were associated with higher incidence of disease, they protected against death, both generally and specifically, once these diseases occurred. At the same time, overweight and obese individuals were estimated to spend more time with disabilities over the course of their lives. As for physical activity, a conscious decision to engage in sports and not to confine one's exercise to a walk around the neighborhood is needed, even at an advanced age, to achieve compression of morbidity towards the end of life. While any type of physical activity was associated with recovery from disability, only vigorous physical activity prevented incident disability. Positive affect, a measure of happiness, was independently associated with protective effects on mortality risk. In participants older than 80 years, this association could largely be explained by the burden of prevalent disease.

3. **Which set of risk factors best predicts death and how do different groups of risk factors compare in their predictive power?**

Age (HR, 95%-CI: 1.09, 1.08-1.10) and female gender (HR: 0.71, 0.62-0.81) were strong predictors of mortality even after adjustment for more than a hundred covariates. Physiologic measures, in particular the risk indicators assessed in blood, such as erythrocyte sedimentation rate, leucocytes, creatinine, C-reactive protein, total cholesterol, or with imaging, such as bone mineral density of the femoral neck and aortic calcification, were all independently related to mortality.

In combination, an individual´s baseline health condition, as measured by prevalent disease, physiologic risk indicators, and general health, was the best predictor of short-term mortality risk. Socio-economic position and lifestyle gave insights into the health risks that developed during follow-up and significantly contributed to the prediction of long-term mortality.

The non-significant findings for the association between single nucleotide polymorphisms and time-to-death and time-to-event, as described in Chapter 2, were disenchanting but curiously still very informative, if seen in relation to other markers of mortality risk as presented in Chapter 4. Although we identified single nucleotide polymorphisms independently associated with mortality, the influence of these markers was indiscernible in the best prediction models. Interestingly, their predictive power seemingly increased with the time span of the prediction.

Methodological Considerations:

Study Design:

All analyses in this paper were executed using data from the Rotterdam Study, a prospective cohort study initiated in 1990 in Ommoord, a district in the city of Rotterdam, the Netherlands. After several extensions of the original cohort, this study currently comprises of observations, interviews, and clinical measures, on 15.000 participants.[1] All the analyses in this thesis were restricted to a baseline population of 7.983 individuals from the first study cohort (78% of 10,215 invitees). The prospective nature of the study design, drawing on a sample from the general population, minimizes selection bias and in combination with meticulous assessment of socio-demographic, lifestyle, indicators of health and disease and genetics allowed meaningful statistical analysis adjusting for a multitude of potential confounders.

Competing Risk:

Independent of the high quality of the data, interval censoring, competing risks, and sample attrition required cautious analyses of the associations of interest. While mortality follow-up was almost complete and right censoring could be addressed by conventional survival analyses, the analysis of disability required a more complex evaluation. Disability in the Rotterdam Study is recorded via interviews which were repeated every 3-4 years. The precise onset of disability or recovery from pre-existing disability is not known. Furthermore, people that died from competing causes of death in between the interview rounds were lost to the disability follow-up. Markov Models (Figure 1), in particular the three-stage illness death model with recovery used in Chapters 3.1 and 3.2, simultaneously addressed competing risks and interval censoring by separately analyzing and accounting for all the possible transitions[2]. Regrettably, selection bias due to informative censoring, i.e. where diseased individuals with a higher likelihood of being disabled generally did not attend the interviews, could not be reduced within this framework.

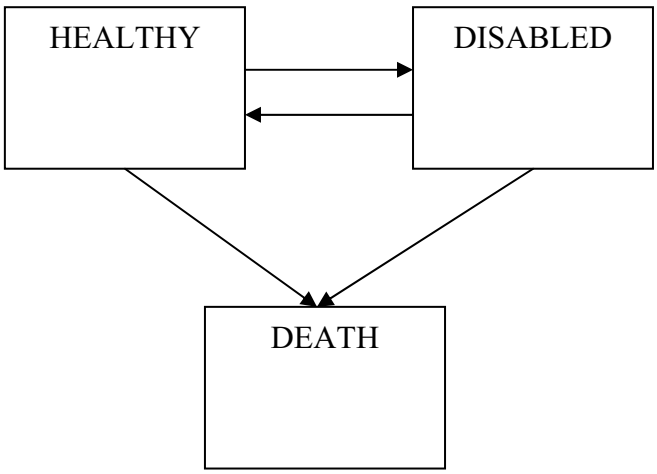

Figure 1 Illustration of the associations considered in a three-stage illness death model with recovery.

Variable Selection:

A consequence of having a large data base was the difficulty in choosing what variables to control for in etiologic analyses. Ideally, all possible confounders are known *a priori* and can then be adjusted for in the analyses.[3] Opinions vary about which variables to adjust for, particularly in regard to confounders. Additionally if several variables that partially control for the same underlying construct are available, conventional analyses cannot accomodate these correlated covariates. Furthermore, variable selection based on hypothesis testing is known not only to result in inflated effect estimates for the determinant, but also to reduce its standard error, leading to

more significant results than advocated by conventional testing theory. These problems of traditional selection algorithms are particularly challenging when the case to variable ratio is low, or the number of covariates even exceeds the number of observations.[1]

In this thesis (Chapter 4), we addressed these problems by using the least absolute shrinkage and selection operator (LASSO), and reporting the results adjacent to the results obtained by backward regression.[4] A comparison of the risk estimates showed that LASSO addressed the inflation of the risk estimates while selecting largely the same variables as the well powered backward regression approach.

Predictive Quality in Time-to-Event Analyses:

For prediction or discrimination analyses, follow-up studies are an essential tool to inform about comparative effect sizes of certain predictors in a specified prediction window. Recent developments lift the restriction on *a priori* defined prediction windows by defining a dynamic risk set at time t composed of cases and controls at that point in time.[5-6] This definition permits the derivation of summary statistics (C-index) for a specific time point and averaged over a defined prediction window.[6]

This approach was used in Chapter 4 to compare different groups of variables and their predictive power over time. Our results clearly illustrated that in general the predictive power of a group of variables decreased with the time horizon that we wanted to predict. This knowledge is important when comparing different prediction studies that use logistic regression and a pre-defined time horizon. It is likely that differences in the prediction window contribute to the disparities found in the literature when predictors are reported and assessed. Interestingly, genetic determinants showed an increasing trend in discriminatory power with longer time horizon. To some, this might mean that genes are actually more important at older age, to others, it might simply reflect the fact that our genes are permanent while other characteristics are subject to changes over time and therefore – if not updated in the prediction model – continuously lose predictive power with longer duration of follow-up. Both options are currently discussed and need to be further investigated.

[1] For a concise overview on the contemporary application of different selection techniques refer to: Walter, S. and H. Tiemeier, Variable selection: current practice in epidemiological studies. Eur J Epidemiol, 2009. 24(12): p. 733-6.

Contribution of our Findings to Ageing Research

When placing our research into context it is important to distinguish between individual ageing, defined as a time-dependent loss of function [7-9], and population ageing, i.e. the shift of the median age of the population to older ages because of declining birth rates and increasing life expectancy.[10] Both of these concepts are linked through the influence of ageing on the life expectancy of the population. Because there is no single biomarker of ageing that can easily be operationalized for analyses at present[11], ageing researchers use different outcomes, starting with death as the most inclusive outcome, longevity as a comparison of specific population subgroups, or composite outcomes such as disease-free survival or disability.

Genetics and Increased Likelihood of Disease and Death

Genes that execute an ageing program have not been identified, and it has been argued that such genes might not exist because there is no evolutionary selective pressure.[7] But genes influencing cellular integrity could certainly influence the probability of survival when adverse environmental conditions are present, and indirectly allow the development of a longevity phenotype.[12] At present, only apolipoprotein E (APOE)[13-15] and human forkhead box O3A gene (FOXO3A)[16-18] have repeatedly been associated with longevity and healthy ageing. But these associations have only been found among centenarians and their siblings. In the general population, such as those that were investigated in chapter 2.2 and to a certain extent in chapter 2.1, these genes could not be identified. Thus while APOE is modestly associated with cardiovascular disease and dementia, it is still unknown whether APOE is associated with survival in the general population.[19-20] The evidence for FOXO3A is not sufficient to allow a similar evaluation.

Several pathways that regulate ageing, such as inflammation, oxidative stress, cellular senescence, DNA damage and repair, and the growth hormone / insulin-like growth factor / insulin axis, have been suggested.[12, 21-23] These and other pathways have been suggested because of their homologues in animal models available from experimental analysis and because of their association to age-related disease and its physiological risk factors.[12, 24-30] Insufficient cellular maintenance, e.g. in the brain, that mediates changes in a broad spectrum of physiological function might also be responsible for ageing.[12, 31] When analyzing longevity, time to death, and time to incident disease or death, we found some indicative evidence for genes associated with cellular maintenance, integrity and neural plasticity.

Nevertheless the limitations of our and other studies need to be considered. It has been argued that any common variation underlying healthy ageing and time to death, as analyzed in Chapter 2.2, has a small effect. Thus it might require even larger sample sizes to identify genetic markers, and our sample size probably limited our ability to discern definite signals. Similarly, there is the likelihood that the heterogeneity of assessing the disease across populations contributed to the disappointing results. Also environmental heterogeneity across studies, birth cohorts and gene-environment interactions require further scrutiny. Lastly, it is also feasible that the heritability of longevity and ageing stems from inherited factors other than single nucleotide polymorphisms analyzed in our studies, e.g. mitochondrial DNA and copy number variations.

Obesity and Physical Activity as Determinants of Disease, Disability and Death

While longevity and healthy aging are heritable, genetic factors influencing ageing cannot be responsible for the recent increase in life expectancy. In this respect it is intriguing that obesity, the phenotype of affluence and a sedentary life style, is repeatedly identified as a double-edged sword. While clearly being a risk factor for potentially fatal disease[32], some studies show longer survival among the elderly obese.[33-38] We argue in Chapter 3.3 that overweight and obesity are beneficial for survival among the non-diseased elderly, and propose a biological explanation that is sensible in times of severe chronic disease. Nonetheless survival bias and the healthy obese phenotype deserve further attention as they could explain our observations. Certainly the results cannot be generalized to younger age groups. There is evidence that overweight and obesity in child- and adulthood reduce survival, and might actually cause future life expectancy to stabilize or even to decrease.[39]

For the purposes of forecasting health care expenditures and pension risks, scientists and policy makers are not only interested in overall life expectancy. Instead, whether or not an individual is healthy or disabled heavily influences their participation in the workforce and the health care resources they will consume. Therefore life expectancy is often divided into disabled and non-disabled life expectancy. These life expectancies give a more informative picture of the health status of the population.[40]

It has long been known that that lack of physical activity and obesity are important risk factors for disability.[41-45] The research presented in this thesis extends this knowledge by showing that obesity in the elderly is not only a risk factor for incident disability, but also for remaining disabled (Chapter 3.2). Because overweight

and obesity were associated with better survival (Chapter 3.3), time spent with disability was significantly increased. There was evidence that females were stronger affected by the disabling effects of overweight and obesity (Chapter 3.2). Being physically active improved survival and reduced the risk of disability. But it was unclear how the type of physical activity influenced the risk of disability. Our research showed that the type of physical activity is important, as the protective effect of physical activity might largely depend on vigorous exercise rather than only on total amount of calories expended (Chapter 3.1).

In summary, the obesity epidemic and an increasing tendency towards a sedentary lifestyle might slow down or reverse the current trend of increasing life expectancy. But even if the increase in life expectancy continues, the obesity epidemic is likely have negative consequences on health care expenditure and pension systems, as overweight and obesity lead to more disabled years of life and poorer health in the population.

Happiness as Indicator of Health and Determinant of Survival

Our research on mental health associated positive affect, i.e. a state of happiness and pleasurable engagement with the environment[46], with beneficial effects on survival. By including well measured confounders we were able to conclude that the survival effect of positive affect is distinct and independent from negative affect, a risk factor for depression. Possible mechanisms can be found in better health behaviors, better health relevant choices over the course of life, and better resilience to infection because of a favorable biological response.[47-49] We also found evidence that among the oldest old the perception of positive affect might largely be defined by prevalent disease, and positive affect ceased to be independently associated with death.

Predicting Mortality - From Gene to Individual Health to Death

Considering that the influence of genetic factors on ageing is ambiguous, what then can genetic factors tells us beyond the traditional risk factors in the prediction of mortality? Although epidemiological research identifies numerous predictors of mortality, information about their comparative effect sizes and long-term predictive power is sparse. The few studies that have evaluated the potential for explaining mortality from a broader perspective, by jointly analyzing demographic characteristics, lifestyle factors and indicators of health and disease, concluded that all of these factors jointly influence mortality. They concluded that these risk factors differ by cause of death[50-51] and that objective, quantitative measures capturing subclinical disease are

superior to clinical history alone for the prediction of mortality[52]. At the same time, the risk factors predicting death are remarkably consistent across countries[53]. We confirmed the superiority of risk factors measured in the blood, and showed that genetic risk factors independently predict mortality. But the contribution of genetic risk predictors in explaining mortality beyond traditional risk factors was negligible.

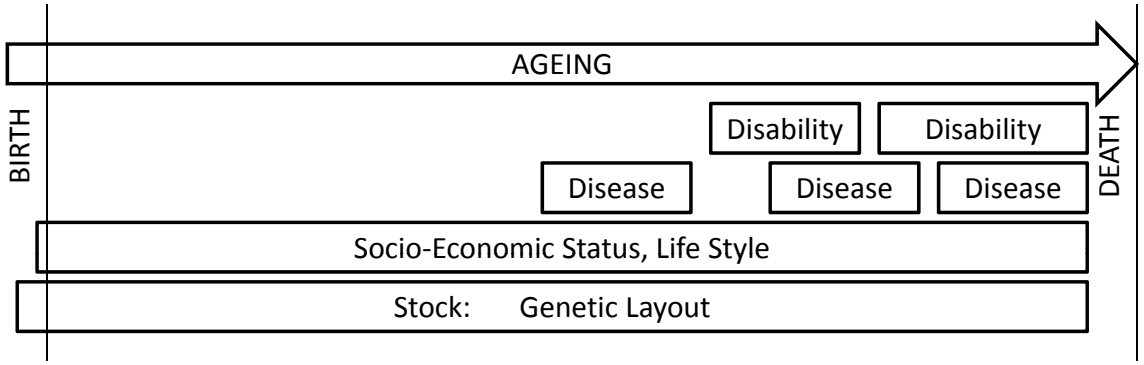

Figure 2 Ageing and the different domains that influence the way an individual experiences ageing.

Finally, summarizing the different studies included in this thesis, one can insinuate a cascade,from gene to individual health to death, in which every step is accompanied by environmental influences, some which are controlled by the individual, such as physical activity and obesity, other defined by the individual´s living circumstances, cultural heritage and surroundings. Figure 2 illustrates at which stage during the course of ageing different interventions, e.g. improvements in living circumstances or the introduction of a new therapeutic drug, can feasibly act and which health gains can be expected. Research is needed to provide unbiased information about the value, defined as health per unit of money spent, of possible interventions.[54] At this stage it is important to consider all the possible health gains till the death of the individual. It is then up to the policy makers to decide which interventions should be implemented after they compete with other societal interests.

Policy Implications

While ageing is no longer a complete mystery, no public health policy explicitly addresses the its negative consequences. Although genetic investigations have delivered clues, they have failed to present a convincing causal framework for the ageing process. But as described above, this definite causal framework is not necessary to address the disease burden in an ageing population. One possibility that is often discussed is to invest in the development of anti-ageing pharmaceutical therapies. Other options include behavioral interventions that promote healthy ageing.

The Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) do not recognize ageing as a treatable entity. Without approval from these bodies, reimbursement opportunities are strongly reduced, and research into biochemical compounds that could postpone the disease burden associated with ageing is financially risky at the very least.[55] Nevertheless there are drugs, rapamycin and metformin, that act on important physiological pathways that have been related to ageing These pharmacological compounds have been developed to suppress the immune system (rapamycin) and as an anti-diabetic drug (metformin) suppressing glucose production in humans. Both address the longevity response to dietary restriction, which is known to extend the life span of mice through the inhibition of TOR (rapamycin) or by stimulating the expression of AMP kinase (metformin).[12, 56-57] Thus they influence a specific ageing pathway but should not be considered anti-ageing drugs.

The drugs described above operate along the longevity response to nutrient intake. Similarly, caloric restriction extends life and health span consistently across species, with supporting evidence from studies ranging from yeast to primates.[58] The reduction of calorie intake is an obvious choice to improve general health and delay the onset of disease in times of the global obesity epidemic.[59] In line with the consequence of overweight and obesity described in Chapters 3.2 and 3.3, an increase in energy intake was associated with an increase in mortality risk when we analyzed predictors of mortality as described in Chapter 4. Similarly, an observational study in elderly Japanese, who during their childhood consumed on average 11% kcal less than usually recommended, confirmed the positive health effects of caloric restriction.[16] It is thus important to recognize that targeting the obesity epidemic would partially influence ageing pathways and lead to improved population health.

Increasing physical activity, as illustrated in Chapter 3.1, is a further example of a behavioral intervention that could lead to compression of morbidity. An increase in physical activity has the potential to at least stabilize, if not improve, physical and cognitive function, and to decrease the number of hospitalizations, chronic disease, and maybe even dementia.[41, 60-61]

Another potentially beneficial strategy operates through increases in social participation. In this thesis we could only confirm independent beneficial effects of happiness and pleasurable engagement with the environment on mortality, as approximated by the association between positive affect and mortality in Chapter 3.4. It

is likely that engagement with the social environment in old age could curb health care expenditures by reducing the incidence of depression and slowing cognitive decline.[10] Facilitating for example communal activities could be a powerful tool for health policy to further improve population health.[10, 62-63]

Future of Ageing Research

Research into ageing is important, because once we completely understand ageing we might be able to prevent age-related diseases and their associated costs, through introducing/developing generalized interventions long before the individual would be considered at risk.[64-65] At present, most health research and policy is targeted towards curing age-related disease or preventing the incidence of a particular condition in specific risk groups. Ageing research promises to prevent or delay several age-related chronic conditions at the same time. To achieve this, the research should focus on improving the health status of the population, i.e. to compress morbidity towards the end of life, and not necessarily to prevent a single disease or extend life span.

Independent of the possibilities offered by the progress in genetic technologies such as DNA sequencing, important scientific progress can be made by better and more rigorous assessment of ageing traits such as disability, disease-free survival, and healthy ageing.[66-68] In many longitudinal cohort studies disability is assessed only as a confounder. This means that it is not measured frequently enough to allow for scientific evaluation of the disablement process and rarely supported by functional measures such as gait speed and grip strength. Disease-free survival requires several diseases to be adjudicated in a population. The adjudication process needs to be repeated constantly for all diseases during the course of follow-up for disease-free survival to be useful as a multivariate phenotype for ageing research. Both disability and disease-free survival, in combination with repeated physiological and functional measures, are necessary to define healthy ageing successfully. Using this data, ageing profiles and trajectories could be developed and used as an outcome in epidemiologic analysis to gain further insights into the underlying biology.[68]

Furthermore, genetic and non-genetic epidemiology should work hand in hand in explaining the causal relationships between risk factors and ageing traits in observational studies. An example for this collaboration are mendelian randomization approaches that aim to use the random allocation of genetic loci and the relationship

between these genes and the risk factors as an instrument to unequivocally confirm or reject hypothesized causal relationship in observational research.[69-71]

Conclusions

In this thesis my co-authors and I investigated different phenotypes of ageing. Although we cannot report a breakthrough in unraveling the genetics of ageing, the brain might play a pivotal role in explaining differences in individual ageing and deserves more scientific investigation. We furthermore show that everyone can improve their personal ageing trajectory through exercise and monitoring their body weight. These findings call for public health action, as they promise to improve population health significantly and thereby ameliorate the pressure on health care budgets and pension systems.

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Chapter 3.3
Walter S, Mackenbach J, Newson R, Hofman A, Tiemeier H. Obesity, incident disease, and mortality – the obesity paradox revisited. Manuscript in preparation.

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Krijthe BP, Walter S, Newson RS, Hofman A, Hunink MG, Tiemeier H. Is Positive Affect Associated With Survival? A Population-based Study of Elderly Persons. Am J Epidemiol 2011; 173(11):1298-307.

Chapter 4.1
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6. Summary

De stijging van de levensverwachting is een belangrijke verdienste van de afgelopen eeuw. Maar de vergrijzing van de bevolking vormt ook een uitdaging voor de maatschappij; de gezondheidszorg en het pensioenstelsel staan onder grote druk omdat de toename van de levensverwachting niet altijd samen gaat met een verhoging van de actieve en gezonde levensverwachting. Een recent rapport van de WHO suggereert dat maatregelen gericht op de obesitas-epidemie, lichaamsbeweging en maatschappelijke betrokkenheid de huidige druk op de gezondheidszorg en pensioenstelsels kunnen verminderen. Druk, veroorzaakt door de nog altijd groeiende hoeveelheid van oudere inwoners, in het bijzonder in westerse economieën. Deze maatregelen zijn er op gericht om verschillende leeftijdsgerelateerde ziekten tegelijkertijd te voorkomen in de populatie. De wetenschap die deze gemeenschappelijke risicofactoren voor leeftijdsgerelateerde ziekten identificeert, stelt beleidsmakers in staat interventies te ontwikkelen die naast een positieve invloed op de gezondheid van de bevolking ook de kosten van de gezondheidszorg verminderen en de druk op het pensioenstelsel verlichten.

Het doel van dit proefschrift is risicofactoren voor functionele beperkingen, ziektevrije levensduur, mortaliteit en een lange levensduur te identificeren. Dit door 1) onderzoek naar genetische loci geassocieerd met een lange levensduur en de levensduur tot ziekte of overlijden, 2) vast te stellen of de Body Mass Index, lichamelijke activiteit, en geluk de tijd tot overlijden en de tijd doorgebracht met een functionele beperking beïnvloeden, en 3) het vaststellen van een verzameling risicofactoren die overlijden het beste voorspellen en hoe deze verschillende groepen van risicofactoren te vergelijken zijn in predictieve waarde.

Hoofdstuk 2 onderzoekt het verband van Single Nucleotide Polymorphisms (SNPs) met een lange levensduur en de tijd tot ziekte of overlijden met behulp van Genome Wide Association Studies. Hoewel de genetische gegevens van 25 000 personen gecombineerd zijn, hebben we geen genetische markers voor veroudering definitief vast kunnen stellen. Voor levensduur, onderzocht met een vergelijking tussen personen die 90 jaar of ouder werden en personen die stierven voor de leeftijd van 80, werd de sterkste associatie waargenomen met rs9664222, $p = 6{,}77 \times 10^{-7}$ na metanalyse met replicatie cohorten. Deze SNP ligt in de buurt van MINPP1 (chromosoom 10), een goed geconserveerd gen betrokken bij de regulatie van cellulaire proliferatie. Wanneer we ons richten op het gezond ouder worden als fenotype en de levensduur tot ernstige ziekte of overlijden, vinden we veertien onafhankelijke SNPs die het risico op overlijden voorspellen en acht SNPs welke ziektevrije overleving voorspellen ($p < 10^{-5}$). Deze SNPs bevinden zich in of bij genen die zich in hoge mate uiten in de hersenen (HECW2, HIP1, BIN2, GRIA1), betrokken zijn bij de ontwikkeling en het functioneren van

het zenuwstelsel (KCNQ4, LMO4, GRIA1, NETO1) en autofagie (ATG4C). Populaire verouderingsnetwerken konden niet ondubbelzinnig worden bevestigd, maar er zijn aanwijzingen gevonden voor netwerken die cellulaire onderhoud en cellulaire dood aan de onderzochte fenotypes, een lange levensduur en de tijd tot ziekte en overlijden, koppelen. Daarnaast is er bewijs gevonden voor associaties van neurale plasticiteit in de hersenen met deze fenotypes.

Hoofdstuk 3 evalueert de invloed van lichamelijke activiteit, Body Mass Index en geluk op sterfte en functionele beperkingen. Obesitas, fysieke inactiviteit, en positief affect beïnvloeden morbiditeit en mortaliteit op verschillende manieren. Overgewicht en obesitas worden geassocieerd met een hogere incidentie van ziekte, maar ze beschermen ook tegen de dood in het algemeen, en in het bijzonder wanneer een ziekte is optreden. Tegelijkertijd wordt geschat dat personen met overgewicht en obesitas meer tijd met een fucntionele beperking door brengen gedurende hun leven dan personen zonder overgewicht. Wat betreft fysieke activiteit, een bewuste keuze om te sporten, en niet zomaar een wandelingetje in de buurt, is nodig om een compressie van morbiditeit te bereiken op oudere leeftijd. Hoewel elke vorm van fysieke activiteit wordt geassocieerd met herstel van ziekte, voorkomt alleen hoge fysieke activiteit het krijgen van een functionele beperking. Positief affect, een maat voor geluk, is onafhankelijk geassocieerd met een negatief effect op het risico op sterfte. Bij deelnemers ouder dan 80 jaar kan deze associatie grotendeels verklaard worden door de ervaren ziektelast.

Het doel van hoofdstuk 4 is het voorspellen van mortaliteit in de oudere bevolking door middel van diverse risicofactoren. De risicofactoren werden als volgt gegroepeerd: leeftijd en geslacht, socio-economische status, levensstijl, algemene gezondheid, aanwezige ziektebeelden, fysiologie en genetica. Leeftijd (HR, 95%-CI: 1,09, 1,08 tot 1,10) en vrouwelijk geslacht (HR: 0.71, 0.62 tot 0.81) zijn sterke voorspellers van mortaliteit, zelfs na correctie voor ruim honderd covariaten. Fysiologische maten, in het bijzonder de risico-indicatoren beoordeeld in het bloed, zoals bloedbezinking, leucocyten, creatinine, C-reactief proteïne en totaal cholesterol, en de risicofactoren beoordeeld met beeldvorming, zoals bot mineraal dichtheid van de femurhals en aorta verkalking, waren allen onafhankelijk van elkaar geassocieerd met sterfte. De combinatie van baseline gezondheid, gemeten met verworven ziekten en fysiologische risico-indicatoren, en de algemene gezondheid is de beste voorspeller van sterfte op korte termijn. Socio-economische status en levensstijl geven inzicht in de gezondheidsrisico's die zich ontwikkelden tijdens de follow-up en leverden daarnaast een belangrijke bijdrage aan het voorspellen van sterfte op lange termijn.

De resultaten voor de associatie van SNPs met een lange levensduur en de tijd tot ziekte en overlijden, zoals beschreven in hoofdstuk 2, zijn hoewel niet significant nog steeds informatief wanneer zij gezien worden in relatie tot andere markers van sterfterisico. Hoewel sommige SNPs onafhankelijk met sterfte geassocieerd zijn, is de invloed van deze markers niet te onderscheiden in de beste predictiemodellen. Interessant genoeg lijkt de predictieve waarde verhoogd te worden met de tijdspanne van de predictie.

Hoofdstuk 5 is een algemene bespreking van het onderzoek zoals samengevat in dit proefschrift. De eerder beschreven onderzoeksvragen worden beantwoord, methodologische uitdagingen bediscussieerd en resultaten besproken in het kader van de uitdagingen voor de gezondheidszorg en het pensioenstelsel. Suggesties voor toekomstig onderzoek, in het bijzonder voor longitudinale studies, worden gedaan en een aantal van de veranderingen die nodig zijn om deze doelen na te streven worden beschreven. De discussie wordt afgesloten met de ontnuchterende constatering dat we geen doorbraak kunnen rapporteren in het ontrafelen van de genetica van algemene veroudering. Maar dat ieder individu wel zijn of haar persoonlijke verouderingstraject kan verbeteren door fysieke activiteit en het toezien op zijn of haar lichaamsgewicht. Deze bevindingen vragen om maatregelen die de volksgezondheid aanzienlijk verbeteren en daarmee de druk op de budgetten voor gezondheidszorg- en pensioenstelsels te verlichten.

The increase in life expectancy over the last century is a major achievement of society. Ageing of the population poses a challenge because health care and pension systems are under pressure as an increase in life expectancy is not necessarily desirable if it is not accompanied by an increase in active and healthy life expectancy. A recent report by the WHO regional office for Europe suggests that measures targeting the obesity epidemic, physical exercise levels, and social involvement could alleviate the current pressure on health care and pension systems due to an ever growing share of elderly inhabitants in particularly in Western economies. These measures have in common that they aim at preventing several disease at the same time in the asymptomatic population. Science that identifies common risk factors for age-associated conditions has the potential to allow policy makers to design interventions that positively influence population health, reduce health care expenditures and alleviate the pressure on the pension system simultaneously.

The goal of this thesis was to identify risk factors for disability, disease-free survival, mortality and longevity by 1) investigating which genetic loci are associated with longevity and time to death and disease, 2) determining whether or not body mass index, physical activity, and happiness influence time to death and time spent with disability, and 3) identifying which set of risk factors best predict death and how different groups of risk factors compare in their predictive power.

Chapter 2 studies the association between single nucleotide polymorphisms (SNPs) and longevity and time to death and disease using genome-wide association studies. Although we combined genetic data of up to 25 000 individuals, we were unable to conclusively identify genetic markers of ageing. When investigating longevity by comparing persons that survived to age 90 and older with persons that died before the age of 80 the strongest association was observed for rs9664222, reaching $p = 6.77 \times 10^{-7}$ after meta-analyses with the replication cohorts. The single nucleotide polymorphism lies in a region near *MINPP1* (chromosome 10), a well conserved gene involved in regulation of cellular proliferation. When turning our attention to the healthy ageing phenotype and counting person years till the onset of major disease or death, we found fourteen independent single nucleotide polymorphisms that predicted risk of death, and eight that predicted event-free survival ($p < 10^{-5}$). These single nucleotide polymorphisms are in or near genes that are highly expressed in the brain (*HECW2*, *HIP1*, *BIN2*, *GRIA1*), or involved in neural development and function (*KCNQ4*, *LMO4*, *GRIA1*, *NETO1*) and autophagy (*ATG4C*). Interestingly, while we could not unequivocally confirm popular ageing pathways, there was some evidence for networks of processes of cellular maintenance and cellular death associated with the phenotypes of time-

to-event and time-to-death. We also found evidence for the association of neural plasticity in the brain with these phenotypes.

Chapter 3 evaluates how physical activity, body mass index, and happiness influence mortality and disability. We showed that obesity, physical inactivity, and positive affect influence morbidity and mortality in various ways. While overweight and obesity were associated with higher incidence of disease, they protected against death in general and specifically once these diseases occurred. At the same time overweight and obese individuals were estimated to spend more time with disabilities over the course of their lives. As for physical activity, a conscious decision to do sports and not only walk around the neighborhood is needed even at an advanced age to achieve compression of morbidity towards the end of life. While any type of physical activity was associated with recovery from disability, only vigorous physical activity prevented incident disability. Positive affect, a measure of happiness, was independently associated with protective effects on mortality risk. In participants older than 80 years this association could largely be explained by the burden of prevalent disease.

Chapter 4 aims to predict mortality in the elderly population by means of a multitude of risk factors. The risk factors were grouped as follows: age and gender, socio-economics, lifestyle, general health, prevalent disease, physiology, and genetics. Age (HR, 95%-CI: 1.09, 1.08-1.10) and female gender (HR: 0.71, 0.62-0.81) were strong predictors of mortality even after adjustment for more than a hundred covariates. Physiologic measures, in particular the risk indicators assessed in blood, such as erythrocyte sedimentation rate, leucocytes, creatinine, C-reactive protein, total cholesterol, or with imaging, such as bone mineral density of the femoral neck and aortic calcification, were all independently related to mortality.

In combination, an individual´s baseline health condition, as measured by prevalent disease, physiologic risk indicators, and general health, was the best predictor of short term mortality risk. Socio-economic position and lifestyle gave insights into the health risks that developed during follow-up and significantly contributed to the prediction of long-term mortality.

The non-significant findings for the association between single nucleotide polymorphisms and time-to-death and time-to-event, as described in Chapter 2, were disenchanting but curiously still very informative if seen in relation to other markers of mortality risk. Although we identified single nucleotide polymorphisms independently associated to mortality, the influence of these markers was indiscernible in the best prediction models. Interestingly, their predictive power seemingly increased with the time span of the prediction.

Chapter 5 is a general discussion of the research summarized in this thesis. In addition to answering the research questions described above, the methodological challenges are addressed and the answers to the research questions are put in the context of the challenges faced by the health care and pension systems described above. Future research directions particularly for longitudinal studies are introduced and some of the changes necessary to pursue these research goals are outlined. The discussion concludes with the sobering finding that we cannot report a breakthrough in unraveling the genetics of ageing, but that every individual can improve his or her personal ageing trajectory through exercise and monitoring his or her body weight. These last findings call for public health action to improve population health significantly and thereby ameliorate the pressure on health care budgets and pension systems.

7. PhD Portfolio

Name: Stefan Walter

Department: Department of Public Health, Department of Epidemiology, Erasmus MC

PhD period: 01.02.2008 – 31.07.2011

Promotors: Johan Mackenbach, Albert Hofman

Co-Promotor: Henning Tiemeier

	Year	**ECTS**
Courses		
Universidad Rey Juan Carlos, Madrid, Spain		
Master Internacional de Formación y Gestion en Medicina Humanitaria (M.Sc)	2009/ 2010	
Humanitarian Action and its Socio-economic Contexts		6
Humanitarian Action		6
Sociology and Economics of Development		6
Management of NGOs and Human Ressources		6
Control of Infectious Diseases		6
Tools for the Implementation, Diagnosis, and Evaluation of Projects		6
Health Care for Conflict Affected and Displaced Populations		3
Humanitarian Action and Development and Cooperation Programs		6
Public Policy and Social Exclusion		6
Netherlands Institute of Health Sciences, Rotterdam, The Netherlands		
Master of Science in Health Sciences, specialization Epidemiology (M.Sc.)	2008/ 2009	
Clinical Trials		0.7
Conceptual Foundation of Epidemiologic Study Design		0.7
Methods of Health Services Research		0.7
Principles of Genetic Epidemiology		0.7
Topics in Health and Diseases in the Elderly		0.7
Large-scale Multicenter Studies		0.4
Clinical Epidemiology		5.7
Methodologic Topics in Epidemiologic Research		1.4
Modern Statistical Methods		4.3
Epidemiology of Infectious Diseases		1.4
Bayesian Statistics		1.1
Psychiatric Epidemiology		1.1
Advanced Analysis of Prognosis Studies		0.9
Analyses of Time-varying Exposures		0.9
Advances in Population-based Studies of Complex Genetic Disorders		1.4
Medical Demography		1.4
Ethnicity, Health, and Health Care		1.1
Ludwig-Maximilians-Universität, Munich, Germany		
Öffentliche Gesundheit und Public Health (M.P.H. postgrad.)	**2007/ 2008**	
Theoretic and Analytical Epidemiology I		3.75
Theoretic and Analytical Epidemiology II		3.75
Biometric and Statistical Methods I		3.75
Biometric and Statistical Methods II		3.75

Socialepidemiology and Medical Sociology		1.8
European Health Systems		1.8
Pharmacoepidemiology		1.8
Epidemiology of Heart Disease3		1.8
Clinical and Economic Decision Analysis		1.8
Medical Informatics		1.8
Epidemiology of Radiation Disease		1.8
Cancer Epidemiology		1.8
Quality Management in Health Care		1.8
Presentation at International Conferences		
"Obesity, incident disease, and mortality – the obesity paradox revisited" Society of Epidemiologic Research (SER), Montreal, 2011	2011	
Teaching activities		
Teaching Assistant in "Principles of Research in Medicine and Epidemiology", Erasmus Summer Program, Rotterdam, The Netherlands	2009/ 2010	
Teaching Assistant in "Methods of Clinical Research", Erasmus Summer Program, The Netherlands	2010	
Reviewer		
European Journal of Epidemiology, PLoS One, American Journal of Public Health, Vaccine, Human Vaccine, European Journal of Human Genetics, BMC Public Health		
Research Stays		
Harvard School of Public Health, Center for Population and Development Studies, "Unemployment insurance policies and the effects of lifecourse income, wealth, and employment status on late life health: disentangling causal effects"	04./05. 2011	

8. Curriculum Vitae

CURRICULUM VITAE

Name:		Stefan Walter
Date of Birth:		22.06.1983
Citizenship	:	German

ACADEMIC MERITS

2008 - 2011	**PhD in Epidemiology and Public Health,** Rotterdam Title: „Determinants of healthy ageing: studies of disability and survival among the elderly". Financed by the Netspar project „Living Longer in Good Health", we investigated risk factors for mortality and morbidity in the ageing population and their impact on society and social services.
2009 - 2010	**Universidad Rey Juan Carlos, Madrid** Management of Humanitarian Interventions (MSc)
2008 - 2009	**National Institute of Health Sciences, Rotterdam** Master of Science in Epidemiology (MSc)
2007 - 2009	**Ludwig Maximilian Universität München** Master of Public Health (MPH)
2005 - 2006	**Friedrich-Alexander-Universität Erlangen-Nürnberg**, Business Studies (Diplom Kfm., 1,7) Focus: Health Management and empirical economic research
2004 – 2005	**Wayne State University**, Detroit, USA Economics (M.A., GPA: 3.79) Focus**:** Economics of Health and Health Care
2002 – 2005	**Friedrich-Alexander-Universität Erlangen-Nürnberg**, Business Studies
2000 – 2002	**Thomas-Mann-Gymnasium**, Lübeck; Abitur, NC 1,4, Germany
1999 – 2000	**Homewood Highschool**, Birmingham, Alabama, USA
1993 – 1999	**Thomas-Mann-Gymnasium**, Lübeck, Germany

EMPLOYMENT HISTORY

09.2011 – present	**Harvard School of Public Health,** Department of Society, Human Development and Health. "Mendelian randomization analysis to establish the causal relationship between anxiety and heart disease."
02.2008 – present	**Panoratio Database Images GmbH**, München Freelance Consultant "Emerging Markets and Health Care"
11.2006 – 01.2008	**Panoratio Database Images GmbH**, München IT consultant implementing longitudinal analyses schemes in health insurance populations. Business development and best practice for statutory and private sickness funds
02.2006 - 07.2007	**Project leader** in the cooperation between ActiveHealth Management, New York and GCN HealthNet, Landshut to promote the implementation of computerized evidence-based decision support systems for general practitioners.
10.2005 - 09.2006	Scientific employee at the **Department of Health Management**, particularly for statistical analyses and evaluations of budget impact calculations.

LANGUAGES

German:	native
English :	excellent
Spanish:	excellent
Italian:	good
Dutch:	good

IT KNOWLEDGE

MS Office

Data Analyses (SAS, SPSS, R-Project, Stata)

Database Management (SQL, XML)

Panoratio Database Image Generator und Explorer (PDI-Technologie)

Dankjewel, Thanks, Danke, Gracias, Grazie, …

This thesis would not have been possible without the support, advice, and comments I received from all my co-authors, collaborators, colleagues, and friends. But first and foremost I want to thank all the participants of the Rotterdam Study. Without your continuous commitment to the Rotterdam Study this thesis would not have been possible.

Thank you!

At the department of Public Health, Erasmus MC and in the AE Gebouw, I would like to thank Liz for being an amazing roommate and reviewing the introduction and discussion of this thesis; James for interesting discussion about the economic crisis; Rasmus for countless attempts (sometimes successful) to motivate me to participate in the cultural life of Rotterdam; Anja, Farsia, and Yolanda for always helping me in solving administrative doubts, arranging appointments, and explaining to me different aspects of Dutch Life; Ewout and Hester for important insights into the world of Prediction and Modeling; Lex for being always among the first people to be at the office and therefore the first point of cheerful reference in many of the rainy Rotterdam mornings. Thanks to the participants of the Intl. Kidz Club in particular to Willemijn and Matejka particularly for introducing me to the Garlic Restaurant LOOK – for all of you who have not been there … best food and service in Rotterdam. I also want to thank all of the people who joined the MGZ walks. These hour long experiences of the Dutch country side were the times I improved my Dutch most. Thanks to all of you for trying my Dutch and not giving up and switching to English.

Stefan, thanks for much of my knowledge in R, Sweave, etc; for the interesting conversations, discussions, and occasional market visits.

Thanks to Akhgar, Sabine, and Claudia for countless, often unexpected dinner invitations that not only increased the variety of nutrient intake by about 100% but also allowed to reflect about the life as a PhD student and sometimes even entirely forget about that very part of our lives.

At the department of Epidemiology, Erasmus MC and the 5th, 21st, and 22nd floor of the EE Gebouw, I would like to thank the members of my Psychiatric Epidemiology working group. Thanks Maris, Karin, Nese, Annemarie, and Fleur for explaining to me the difference between Cortisol and Cholesterol and for the coffees, cookies, dinners, and rejuvenating (just to refer back the importance of ageing research) PsychEpi group meetings in the Valkenburg Library. Thanks Bouwe for allowing me to use your article on positive affect and mortality in this thesis and for writing up the results so nicely that we could publish it in AJE. Thank you Rachel for challenging my thinking and approach towards science and the Irish Pub Quiz Nights. Thank you Rachel for teaching me the colloquial Dutch language and Alex and Karolina for sharing many a moment laughing at the pit-falls that these colloquial can mean to someone with German as a first language.

I would like to thank Cornelia, Arfan, Janine, Mark, Maksim, Karol, Aaron, Yurii, Ayze, Linda, Abbas, Suman, and Maryam for interesting discussions and help particularly regarding the genetic parts of this thesis. It is a luxury to have so much knowledge - be it epidemiological, technological, or just simply scripts - around you when you try to finish a paper in the world of GWAS.

I would also express my special thanks towards the CHARGE collaborators. This new world of collaborative science is amazing. I appreciated all your inputs to the two genetic manuscripts reproduced in this thesis. In particular I would like to thank Joanne, Anne, Gil, Kathy, Bruce, Greg, Nora, and Melissa. Thank you for actively writing and rewriting the manuscripts.

A special thanks goes to Jan who explained the Dutch health care system to me and facilitated access to the same tremendously. Thank you! And of course to Bruno who allowed me to stay in his corner office for large part of my time at Erasmus.

I also want to thank Hettie and Jacqueline who facilitated all the administrative tasks that surround a PhD students life in the department of epidemiology. I would also like to thank the HR department in particular Solange and Andreas for facilitating my stay with the correct information and directing me through the jungle of obtaining my SoFi Nummer, Health Insurance, and the Personal Budget.

Thanks to the IT; to Nano for discussing the newest gadgets and for finding creative solutions to most of my IT problems, to Frank for answering to absolutely every question about the Rotterdam Study data, and to all the others from the IT corner room for jointly soothing me when unexpected IT problems arose.

I would like to express my thanks and gratitude jointly to Johan, Bert, and Henning for giving me the chance to obtain my PhD under their auspices at the Erasmus MC in Rotterdam.

Johan, thank you very much for leading by example. The meetings with you will always be remembered as the most efficient, most challenging, and most productive.

Henning, thank you very much for always being there. If not physical then definitely by email. Agreeably you had the most difficult task of all by having a PhD student like me who quiet unconventionally defined his contribution TOTALLY not by being at the office between 9 – 5. Who at the same time is a friend and that demanded justification for absolutely everything. I think we managed quite well.

Bert, thank you very much for inciting passion and motivation. From the first presentation of yours at the LMU in Munich, to the discussions about where to send our work, and more importantly how to present it.

A special thanks goes to David, for the first time I understood the power of words. Special thanks also to Sabine from the LMU. Thank you for opening the path to Public Health by permitting me to work and study simultaneously.

Azi and Ruth, thank you very much for the Summer Program 2008. The discussions about the Bloody Spanish, the Axis of Evil, and life and love in general will always be remembered by me.

Ein Dank an meine Familie. Siggi und Horst, Oma und Opa, vielen Dank! Ihr seid immer da, wenn man Euch braucht. Immer. Arbeit, Ehrlichkeit, Fairness, … und vieles mehr habt Ihr mir vorgelebt. Danke. Anneliese und Hans – Danke. Jochen, wir haben mehr gemeinsam als uns lieb ist, aber auch Du hast mich zu dem gemacht der ich bin. Danke. Andrea, Mutti – hier sei es mal gesagt - danke für ALLES. Du machst einen super Job als Mutter, Chef, und Freund und bist ein wahres Vorbild für mich und viele andere. Justus, ich bin beeindruckt und gespannt. Danke, dass Du in Lübeck die Fahne hochhältst und mir ab und an die andere “normale” Welt

zeigst. Charlie, noch eine Chemikerin ;-) – Glückwunsch! Danke fürs Zuhören und viele, tolle Tage insbesondere in Madrid. Stefan, vielen Dank, dass Du mich immer begleitet hast. Einen besseren PatentOnkel kann man sich nicht vorstellen. Dank auch an Dich Uta, dafür dass Du diese Gespräche mit noch einer weiteren Sicht der Dinge bereicherst. Und an Eliseo und Aeneas – Danke. Ich weiss wir sehen uns viel zu wenig, aber wenn wir uns sehen haben wir ne gute Zeit zusammen. Vielen Dank Brigitte für die nüchterne Sicht auf viele Dinge.

I have the privilege of having several families. So here is the place to thank all of those living in Alabama. Chris, Belinda, Chip, Cady, Charly, Joey, Brody, Rocky thanks for always welcoming me back home. Thank you Pina and Sam for memorable moments in Haleyville.

A mi familia mexicana. Gracias Tichi, que nuestra amistad siga siendo igual de fuerte en el futuro. Beatriz, Carlos, Carlos, Rafa muchas gracias por enseñarme lo que es México. Estoy feliz de haber encontrado una familia igualita a la mía de Alemania allí en el D.F.

Y también a mi familia española. Gracias Fabio y Tita por darme la bienvenida en vuestra casa. Es un placer vivir en Madrid y compartir muchas comidas riquísimas con vosotros. Me siento en casa. Gracias también a mis cuñaaaaaaos por enseñarme el español de verdad. Gracias a Andrés por la cerveza alemana, los chuletones de Los Picapiedra y por diseñar la portada de esta tesis. Gracias a Ángel por los intercambios científicos. Gracias a las abuelas por tratarme como un nieto más. Y por supuesto a Maribel y Juan por las croquetas exquisitas que me guardan siempre.

Ruth, no hay palabras suficientes para agradecerte. Because I would always miss out on a couple of the important ones. Ich liebe Dich und bin dankbar dafür mit Dir eine gemeinsame, unsere gemeinsame Zukunft gestalten zu dürfen.

a la mariposa blanca sobre fondo gris