How Genes Modulate Patterns of Aging-Related Changes on the Way to 100: Lessons from Biodemographic Analyses of Longitudinal Data

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Presented at the Living to 100 Symposium

Orlando, Fla.

January 8–10, 2014

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Introduction

Despite the high potential of available data and evident progress in clarifying the genetic nature of many complex traits, there are limitations to genome-wide association studies (GWAS) in the genetic analyses of life span and other longevity-related traits. Several factors could be responsible. Decades of studies of candidate genes show that new conceptual ideas are needed to better understand genetic mechanisms involved in regulating aging-related traits.[1] Recent GWAS provide additional evidence about the need to develop better methods to understand the complex nature of such traits.[2] The weakness of theoretical concepts about the nature of late-life traits, including difficulties in evolutionary explanations of research findings, is another reason for slow progress in the research field.[3] The pleiotropic and age-dependent genetic associations detected in recent genetic studies still do not have adequate conceptual descriptions.[4]

The inherent complexity of gene actions on traits in late life can well explain why many genetic signals appear to be weak. Indeed, weak effect of genes on traits in late life can be not only because they confer small risks but because they might confer large risks but in a complex and dynamic fashion.[5] Accordingly, aging-related processes can be the key to better understanding the nature of weak genetic effects and, consequently, the genetic origin of health span and life span.

Most GWAS and subsequent calculations are not based on analyses of the biology of genetic influence on such traits, and often do not include the following points:

- many genetic and nongenetic factors contribute to such traits;
- contribution of specific genes depends on genetic background (internal milieu created by other activated genes), which, in turn, can be modulated by external conditions; and
- genetic effects are mediated by many biological variables that change their values and their influence on aging and longevity traits during the life course.

The strategy of considering one single-nucleotide polymorphism (SNP) at a time with subsequent evaluations of the effects of separate genetic variants on traits of interest has resulted in a "missing heritability" problem.[6] The proportion of phenotypic variance of complex traits explained by genetic factors found in GWAS is much smaller than expected based on estimates of these traits' narrow-sense heritability evaluated in the pregenomic era.

Most researchers studying the genetics of human longevity search for genes that contribute to increased longevity. This strategy ignores the possibility that exceptional longevity could result from the absence of many harmful genetic factors which contribute to premature death, especially for individuals having a large number of such "frailty" alleles or genotypes. The presence of many such frailty genes has been hypothesized in the

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[1] Finch and Tanzi, "Genetics of Aging," 407–11; and De Benedictis et al., "Recent Advances," 909–20.

[2] Teslovich et al., "Biological, Clinical and Population Relevance," 707–13; and Yashin et al., "Polygenic Effects," 381–94.

[3] Di Rienzo and Hudson, "Evolutionary Framework," 596–601; and Vijg and Suh, "Genetics of Longevity," 193–212.

[4] Kulminski et al., "Polymorphisms," 13–21; Summers and Crespi, "Xmrks the Spot," 3022–24; Williams and Day, "Antagonistic Pleiotropy," 1478–88; Kulminski and Culminskaya, "Genomics," 455–69; and Yashin et al., "Have the Oldest Old Adults," B432–42.

[5] Kulminski and Culminskaya, "Genomics," 455–69; Ukraintseva et al., "Trade-Offs Between Cancer," 387–96; and Kulminski et al., "Beta2-Adrenergic Receptor," 338–45.

[6] Gibson, "Hints of Hidden Heritability," 558–60; Lee et al., "Estimating Missing Heritability," 294–305; Manolio et al., "Finding the Missing Heritability," 747–53; Slatkin, "Epigenetic Inheritance," 845–50; Visscher, Hill, and Wray, "Heritability in the Genomics Era," 255–66; Makowsky et al., "Beyond Missing Heritability," e1002051; Eichler et al., "Missing Heritability," 446–50; and Zuk et al., "Mystery of Missing Heritability," 1193–98.

mutation accumulation theory of aging.[7] A number of detected variants show pleiotropic effects on disease traits and dynamic characteristics of aging-related changes taking place during the life course.

The conventional methods for genetic and nongenetic analyses of data on longevity-related traits underutilize available information on aging, health and life span. Most of these methods just ignore the available knowledge about the traits of interest and treat the limited data set as the only source of information about the traits. This practice misses the opportunities presented in the research potential of the available data.[8] It inhibits systemic integration of available information on aging and longevity, and slows down the progress in improving our understanding of either the nature of these traits or factors affecting them. The power and biological relevance of GWAS can be enhanced by incorporating the biological and demographic principles of trait formation into the backbone of genetic analyses, through appropriate mathematical models and statistical functions, and by incorporating genetic questions into a comprehensive framework of dynamic analyses of longitudinal data. This can be done by incorporating hidden biomarkers characterizing stress resistance, adaptive capacity, physiological norms, and effects of allostatic adaptation and allostatic load into a dynamic stochastic process model (SPM) of human aging, health and longevity and by using this model in statistical analyses of genetic, static nongenetic and phenotypic longitudinal data. The model appropriate for such analyses, SPM, has been developed and validated in the studies of subsets of the longitudinal data.[9] The use of the genetic version of such a model (GenSPM)[10] allows us to synthesize all of the components and the outcomes, and to evaluate how genetic effects on aging, health and the longevity traits are mediated by physiological indices and the key biomarkers of aging. Note that an important advantage of using the genetic version of the stochastic process model of human aging, health and mortality is the opportunity to study roles of genetic factors in hidden biomarkers of aging and their connection with health and survival outcomes. These biomarkers (stress resistance, adaptive capacity, effect of allostatic load, allostatic adaptation, physiological norm) are considered in the model as a part of the biological mechanism involved in forming partly observed age trajectories of physiological indices as well as risks of health and survival outcomes. Such analyses allow for testing whether age trajectories of biomarkers of aging depend on the individual's genetic background and whether parameters describing age patterns of mortality rates as well as other hazard rates differ for individuals with different genetic background.

The Framingham Heart Study

The Framingham Heart Study (FHS) includes 14,428 participants, from whom 9,215 were genotyped for 550,000 SNPs. The FHS original cohort was launched in 1948 (exam 1), with 5,209 respondents (55 percent females) age 28–62 residing in Framingham, Mass., who had not yet developed overt symptoms of cardiovascular disease[11] and continued to the present with biennial examinations (30 exams to date) that include detailed medical history, physical exams and laboratory tests. The offspring cohort (FHSO) was launched in 1971 (with eight exams to date) with 5,124 second-generation individuals (52 percent females), who are the original FHS participants' adult children and their spouses.[12] The third generation cohort, consisting of the

[7] Albin, "Antagonistic Pleiotropy," 279-86; and Charlesworth, "Patterns of Age-Specific Means," 47–65.

[8] Yashin et al., "Genes, Demography, and Life Span," 1178–93; Yashin et al., "Genes and Longevity," B319–28; Yashin, Arbeev, and Ukraintseva, "Accuracy of Statistical Estimates," 243–55; Arbeev et al., "Evaluation," 157–66; and Yashin et al., "How the Quality of GWAS."

[9] Yashin et al., "Stochastic Model," 538–51; Yashin et al., "Model of Hidden Heterogeneity," 1–10; Yashin et al., "What Age Trajectories," 191–200; Yashin et al., "Cumulative Index," 75–86; Yashin et al., "Health Decline," 291–302; Yashin et al., "Maintaining Physiological State," 611–18; Yashin et al., "Exceptional Survivors," 257–65; Yashin et al., "Joint Analysis," 207–33; Yashin et al., "New Approach," 5336–50; Yashin et al., "Quadratic Hazard Model," 177–88; Yashin et al., "Patterns of Aging Related Changes," 403–33; Yashin et al., "How Lifespan Associated Genes"; Arbeev et al., "Genetic Model," 103–11; Arbeev et al., "Age Trajectories," 93–102; and Arbeev et al., "Effect of the APOE Polymorphism."

[10] Arbeev et al., "Genetic Model," 103–11.

[11] Dawber, Meadors, and Moore Jr., "Epidemiological Approaches," 279–86.

[12] Kannel et al., "Investigation of Coronary Heart Disease," 281–90; and Splansky et al., "Third Generation Cohort," 1328–35.

grandchildren of the original cohort participants, having at least one parent in the offspring cohort, totaling 4,095 individuals (53 percent females), was added to the study with the first examination completed in 2005.[13] The three FHS cohorts use similar research protocols so comparisons could be made. Across the three generations, 99.7 percent of participants are white.

Phenotypic traits collected in the FHS cohorts over 60 years and relevant to our analyses include:

- life span;
- cause of death;
- age at onsets of cardiovascular diseases (CVD), cancer and neurodegenerative disorders (ND);
- indices characterizing disease and recovery progress (blood, urinary, mental and physical tests; use of medication and other treatment);
- internal and external disease risk factors, including diastolic blood pressure (DBP), systolic blood pressure (SBP), ventricular rate (VR), blood glucose (BG), serum cholesterol (CH), body mass index (BMI), and demographic, behavioral and life history characteristics; and
- selected markers of aging.

The occurrence of CVD, cancer, ND and death has been followed through continuous surveillance of hospital admissions, death registries, clinical exams and other sources, so that all the respective events are included in the study.

FHS genetic data includes 9,215 individuals from three generations of the FHS who were genotyped for genome-wide SNPs, with results available through the Framingham SNP Health Association Resource (SHARe). The genotyping was conducted using Affymetrix platform with about 550,000 SNPs representing a significant part of human genome variability. Individual information is publicly available through the Framingham SHARe upon request. From the FHS and FHSO generations, 5,182 individuals have information on the apolipoprotein E (*APOE*) e2/3/4 polymorphism. In this paper we discuss the results of analyses of data from the original FHS cohort for genome-wide data and from the FHS and FHSO cohorts for the candidate-gene data. First we discuss effects of *APOE* e4 carriers and noncarriers on survival and probabilities of having CVD, cancer (but not skin) and neurodegenerative diseases.

APOE and diseases in late life

Here we consider the effects of the *APOE* e4 (e2/4, e3/4 and e4/4) allele contrasted by the non-e4 allele (e2/2, e2/3 and e3/3) genotypes on the risk of major human diseases including CVD, cancer and ND. Figure 1 demonstrates age patterns of the probability of remaining free of CVD, cancer and ND for carriers and noncarriers of the e4 allele in the FHS original and offspring cohorts in each gender. These patterns reveal the complex role of the e4 allele in risks of certain diseases, which may be different at different ages, generations and genders.

[13] Splansky et al., "Third Generation Cohort," 1328–35.

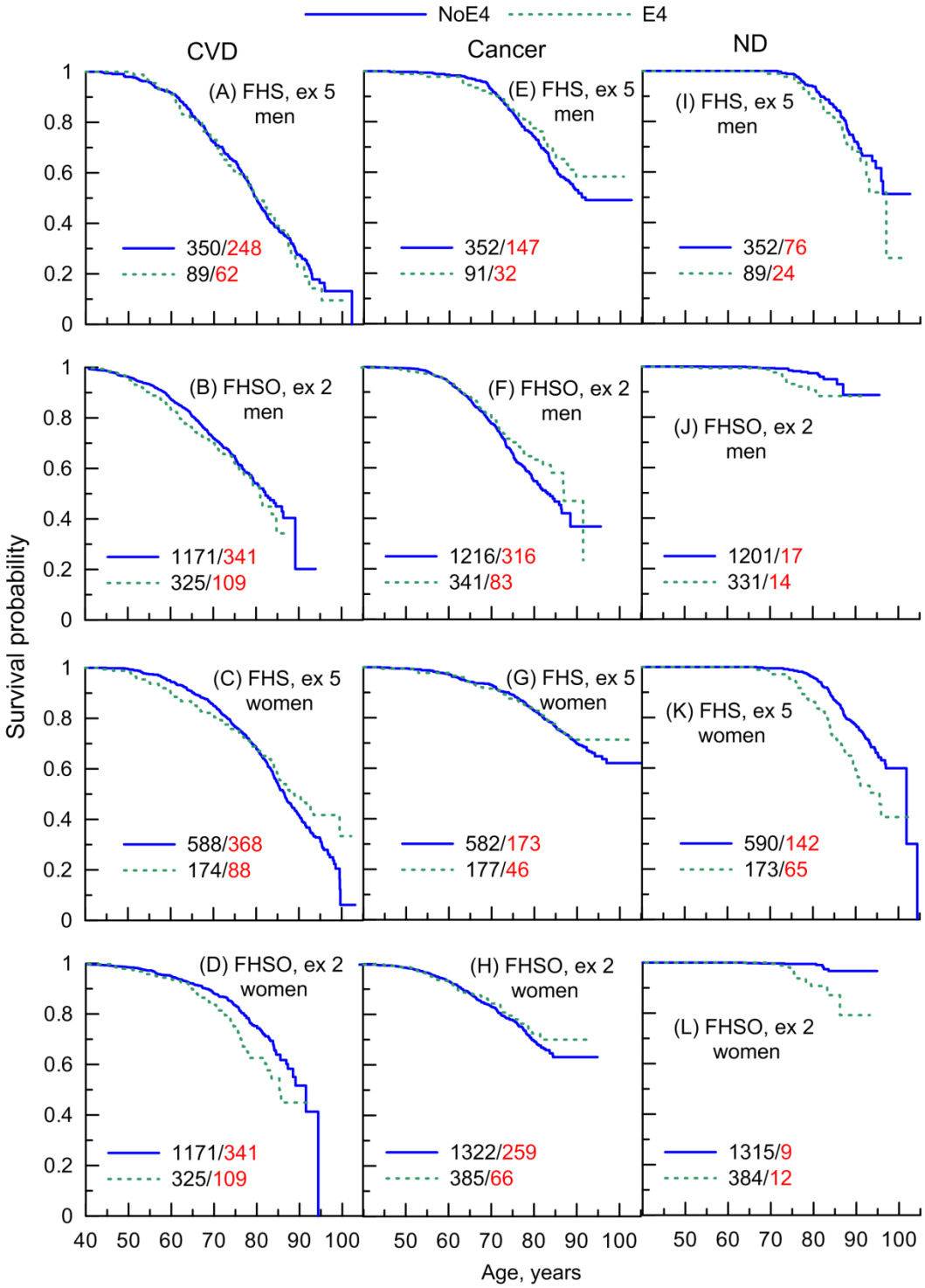

Figure 1. Empirical age patterns of the probability of staying free of cardiovascular diseases (left column), cancer (all sites but skin; middle column) and neurodegenerative disorders (right column) for carriers (E4) and noncarriers (NoE4) of the *APOE* e4 allele who attended the fifth examination in the original FHS cohort and second examination in the FHSO (offspring) cohort. N = m/k denotes (m), the total number of carriers, and (k), the number of disease cases among them. Censored individuals are not depicted.

The e4 allele does not affect the risks of CVD in men regardless of generation; however, the allele affects risks of cancer and ND. The effects of this allele are of the same direction in each generation for cancer and ND. When the allele confers risks of ND but protects against cancer, we observe genetic tradeoff. Analysis of age patterns for cancer and ND shows the effect of the e4 allele tends to be more pronounced in the offspring cohort, and analysis of age patterns for cancer also reveals the protective effect is characteristic for cancer with onset at older ages.

For women, the e4 allele affects the risks of CVD but does not affect the risks of cancer, regardless of generation. Similarly to men, the allele confers risks of ND for women in each generation. The effects of the e4 allele on CVD are sensitive to age; in the FHS original cohort, we observe that the effect changes its direction from detrimental for onset at younger ages to protective for onset at older ages. In the offspring cohort, we observe detrimental effect of the e4 allele on CVD primarily at older ages.

To quantify these empirical observations, we conducted Cox regression analyses and evaluated the risks of CVD, cancer and ND. Given the disproportionality of hazards seen in figure 1, such as age-sensitivity in the effects of the e4 allele on risks of CVD and cancer, we selected more homogeneous samples of individuals who contracted diseases in a given age period characterized by proportional hazards. Then we evaluated the risks of CVD and cancer in these more homogeneous groups and the ND risks in all the samples. To ensure these estimates are robust to longitudinal attrition of individuals at risk of these diseases, we evaluated the risks considering different examinations as baselines. Figure 2A shows that the observed effects are stable regardless of attrition of the sample of individuals at risk for these diseases.

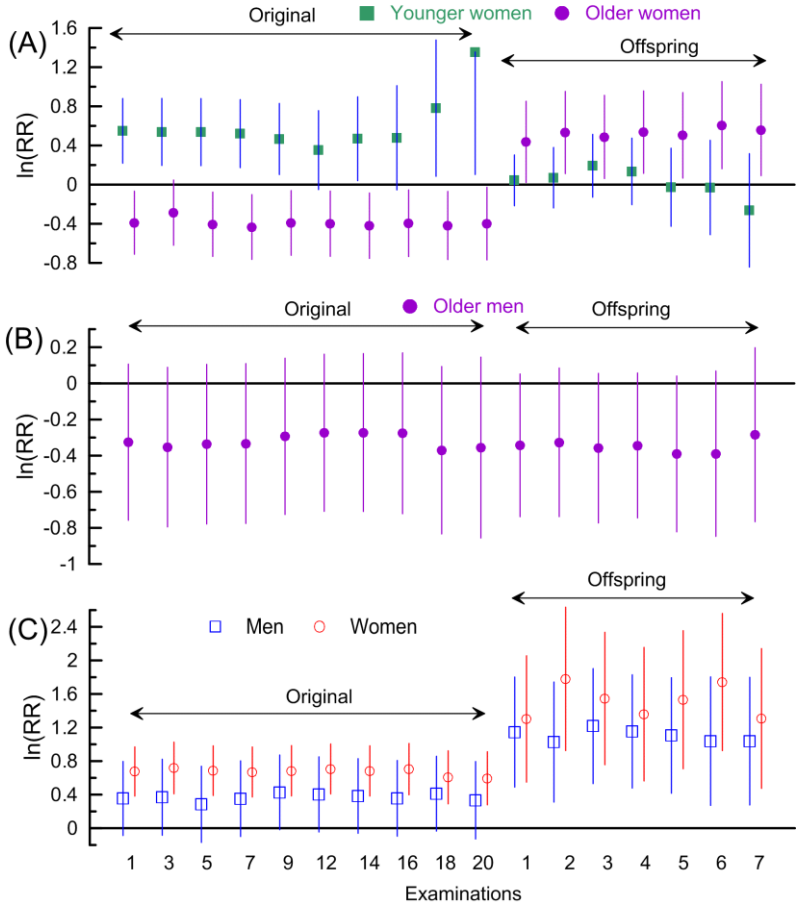

Figure 2. Natural-log-transformed relative risks (RR) of (A) CVD, (B) cancer and (C) neurodegenerative disorders in the *APOE* e4 allele carriers compared to the non-e4 carriers at different examinations in the FHS original and offspring cohorts. The RRs were evaluated in representative, more homogeneous groups as follows: (A) women, ≤75 years at onset or censoring (younger) and >75 years at onset or censoring (older) in the FHS as well as <70 years at onset or censoring (younger) and ≥70 years at onset or censoring (older) in the FHSO, and (B) men ≥70 years at onset or censoring in the FHS and the FHSO. The estimates in (C) for men and women are for the entire samples. Thin bars show 95 percent confidence intervals; the negative direction at examination 20 in (A) is shown for the sake of better resolution.

Figure 2A shows that women from the younger group carrying the e4 allele in the FHS and from the older group in the FHSO are at increased risk of CVD. Women from the older group carrying the e4 allele in the

FHS appear to be protected against CVD. No effect of the e4 allele is observed in women from the younger group in the FHSO. Figure 2B shows that men from the older group carrying the e4 allele tend to be protected against cancer. Figure 2C shows that both men and women carrying the e4 allele are at higher risk of ND.

Figures 2A–B show that straightforward strategies on revealing genetic effects by increasing sample size may either not work at all (figure 2A) or be inefficient (figure 2B) because of the inherent heterogeneity of the age-related traits. Specifically, increasing sample size by pooling data from the FHS original and offspring cohorts and disregarding the age-related heterogeneity shows no significant effects of the e4 allele on risk of CVD or cancer (table 1). However, the lack of effect in the case of CVD is not because the e4 allele has no effect on CVD but because this effect is complex, with the allele playing protective and detrimental roles at different ages and in different generations (figure 1, left column). Taking into account that the e4 allele is associated with cancer only at older ages, the estimate of the risk in the pooled sample of older men (representatively, those who attended the fifth examination in the FHS original cohort and second examination in the FHS offspring cohort) becomes significant: RR = 0.72, p = 0.032 (95% CI = 0.53–0.97).

Table 1. Relative risks of CVD, cancer and ND in the pooled sample of genotyped participants who attended the fifth examination in the FHS original cohort and second examination in the FHS offspring cohort.

Gender	Disease	RR	p	95% CI
Women	CVD	1.04	0.691	0.87–1.25
Men	Cancer	0.84	0.095	0.68–1.03
Women	ND	2.22	2.0×10^{-8}	1.68–2.94
Men	ND	1.63	0.012	1.11–2.38

Note: The number of subjects is shown in figure 1A; RR = relative risk; CI = confidence interval.

Figures 1–2 demonstrate important results highlighting three modes of gene actions on traits in late life. The first is illustrated by the associations with CVD, which are observed primarily in women. Differences in the effects across ages (older and younger groups in the FHS) and generations (the original and offspring cohorts) imply that mechanisms linking the e4 allele with CVD in women are likely sensitive to environmental changes occurring during the life spans of the original and offspring generations. The second is illustrated by the associations with cancer primarily in men. Specifically, we observe the remarkably sustainable effects of the e4 allele on cancer across generations, which are limited to the same old ages. This observation implies a much more sustainable mechanism linking the e4 allele with cancer, which is weakly sensitive to recent environmental changes. Because these effects are observed at the same old ages but in different generations, it is likely this mechanism is relevant to the aging process in men. The third is the conventional age- and generation-insensitive effect of the e4 allele on ND in each gender (albeit it is more pronounced in women).

Genotyped and nongenotyped individuals in the original FHS cohort. In addition to data on specific genes such as *APOE* and others, the Framingham study collected data appropriate for genome-wide association analyses. Using these data, we investigated associations of genetic variants with aging and longevity in the original FHS cohort. It is important to note that only 1,471 out of 5,209 individuals from the original FHS cohort were genotyped to get information on 550,000 SNPs. To understand the difference between genotyped and nongenotyped individuals of the original FHS cohort, we compared survival functions and age trajectories of physiological variables for these groups of individuals. Figure 3A shows the average age trajectories of eight physiological indices for genotyped (G) and nongenotyped (NG) females.

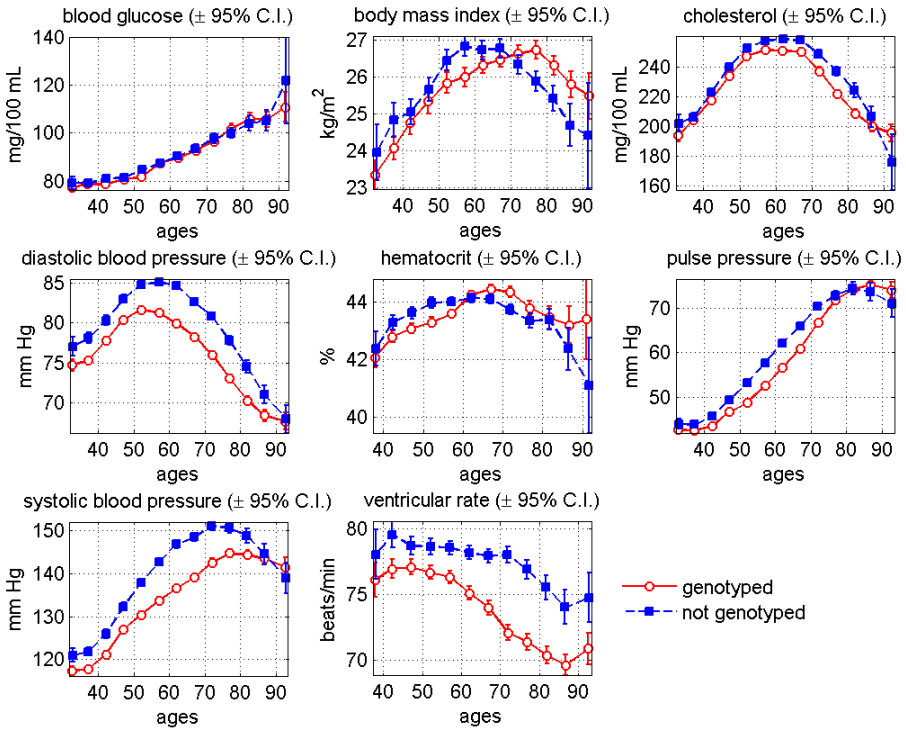

Figure 3A. Age trajectories of eight physiological indices including blood glucose, body mass index, cholesterol, diastolic blood pressure, hematocrit, pulse pressure, systolic blood pressure and ventricular rate for genotyped and nongenotyped females in the original cohort of the FHS data.

One can see from this figure that the age trajectories of BG were about the same for genotyped and nongenotyped females. The values of BMI were slightly higher among NG females until age 70 and then they become lower. The values of DBP and VR are higher among NG females. The values of CH and SBP were slightly higher among NG females until age 85 and became indistinguishable after this age. The values of pulse pressure (PP) among NG females were higher until age 75 and became indistinguishable after this age. The values of hematocrit (H) tend to be higher in NG females until age 60 and then become lower than those of G females, although the difference is small and became indistinguishable after age 80.

For males (figure 3B), the difference between average age trajectories of BG, DBP, CH, H and VR for genotyped and nongenotyped individuals were about the same as for females. However, the BMI values were about the same for NG and G males until age 50. Then BMI goes lower for NG than for G males. The values of SBP and PP for NG and G males became indistinguishable after age 75. As nongenotyped people presumably are those who died at earlier time periods, the difference between curves also reflects contribution of over-time trends in age trajectories of physiological indices.

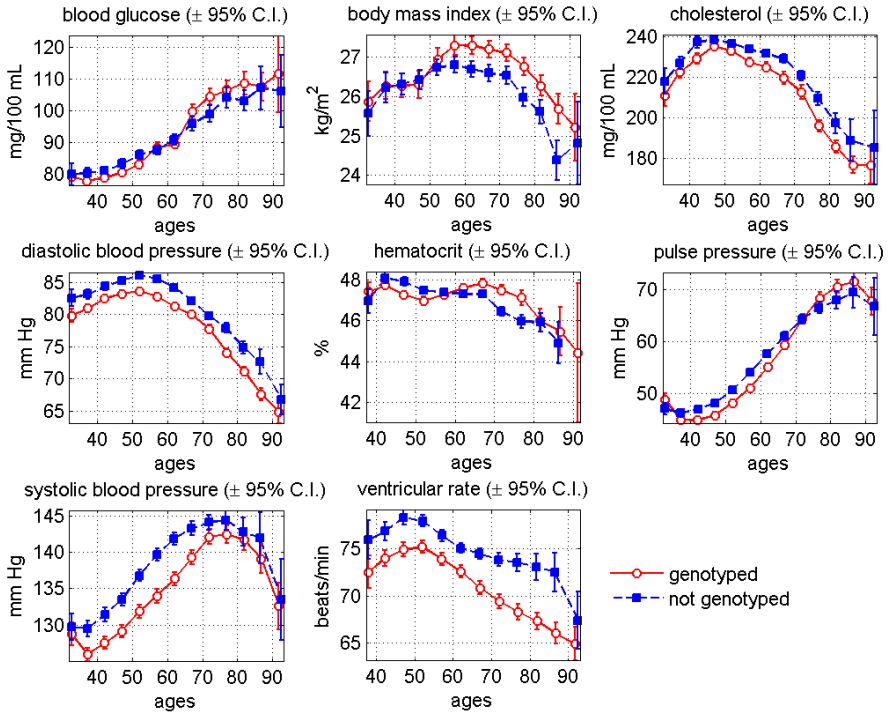

Figure 3B. Age trajectories of eight physiological indices including blood glucose, body mass index, cholesterol, diastolic blood pressure, hematocrit, pulse pressure, systolic blood pressure and ventricular rate for genotyped and nongenotyped males in the data from the original FHS cohort.

Survival for genotyped and nongenotyped individuals. Figure 4 shows four survival curves conditional on survival to ages 65 and 80 for genotyped and nongenotyped females (left panels) and males (right panels) starting with age 65 (top panels). One can see from the top panels of this figure that conditional survival functions for nongenotyped males and females survived age 65 were higher than those of genotyped individuals starting from age 68. The curves became indistinguishable at age 87 for males and at age 90 for females. It is interesting that conditioning on survival to age 80 substantially changes this picture (bottom panel). The conditional survival curves at age 80 for males and females for genotyped and nongenotyped individuals stay about the same until age 85. Then curves for genotyped individuals diverge from those of nongenotyped individuals showing worse survival. The curves converged at about age 100.

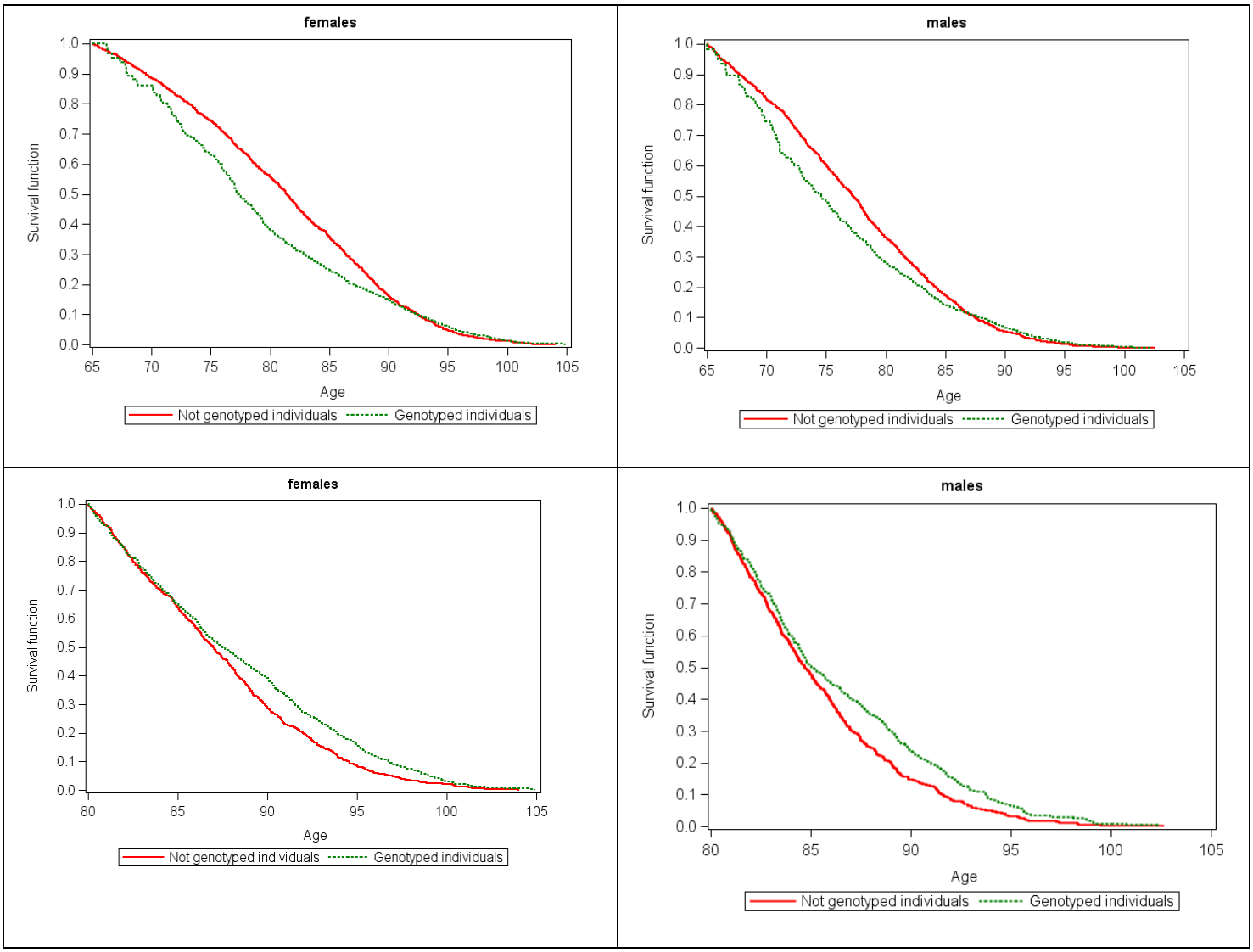

Figure 4. Survival functions of genotyped and nongenotyped male and female participants of the original Framingham cohort conditional on survival until 65 years (top panel) and until 80 years (bottom panel).

Note that although it is clear that differences in age trajectories between genotyped and nongenotyped individuals shown in figures 3 and 4 somehow reflect the genotyping strategy and over-time trends, the specific factors responsible for this difference are still not well understood. This indicates that conclusions based on studying only genotyped individuals cannot be extended to the entire population of the original FHS cohort.

Average age trajectories of genotyped individuals for males and females.

Figure 5 shows that the age trajectories of BG and PP were about the same for males and females, with a slight difference between ages 65 and 80 for BG and for PP before age 45, where female PP values were lower, and after age 70, where female PP values were higher. The BMI values for females were lower than that of males until age 75. After this age, the curves practically coincide. The values of CH were lower for females until age 45 and then became higher until the end of the observation interval. The values of DBP were lower in females until age 75. After this age, the curves became indistinguishable. The SBP was lower for females until age 50. Then the males and females curves practically coincide until age 75. After this age, the female SBP curve became higher than that for males. The H curve for males is higher than that of females for the entire interval, and the VR curve is higher for females for the entire age interval. It is clear that the observed

difference between males and females is partly of genetic origin. It is also likely that males and females experienced different exposure to external conditions.

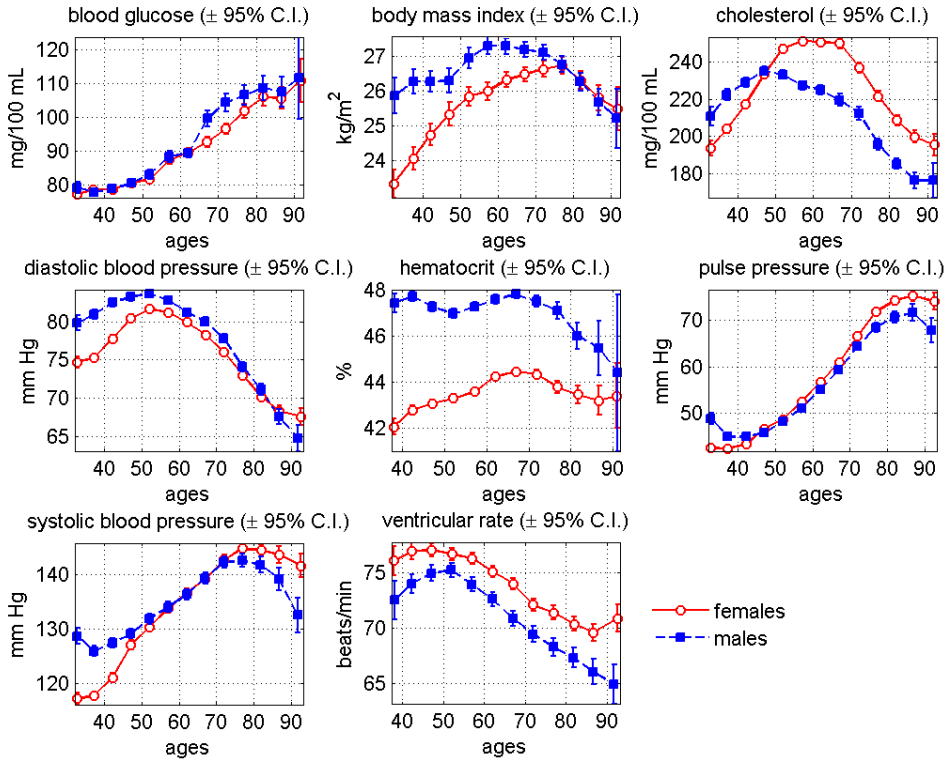

Figure 5. Age trajectories of eight physiological indices including blood glucose, body mass index, cholesterol, diastolic blood pressure, hematocrit, pulse pressure, systolic blood pressure and ventricular rate for genotyped males and females in the data from the original FHS cohort.

Age trajectories of standard deviations of eight physiological indices for genotyped males and females

Standard deviations (SD) of the value of physiological variables at a given age characterize the variability of values of corresponding variables among study participants at this age. Figure 6 shows age trajectories of standard deviations of eight physiological indices for genotyped males and females. One can see from this figure that for DBP, H, PP, SBP and VR, these SDs are about the same among males and females. However, for BG males, SD was higher between ages 65 and 80. For BMI, SD was higher for females than for males starting from age 40. For CH the values of SD for females were lower than those of males until age 45, and then they became higher and remained higher until age 85. After these ages, the values of SD became indistinguishable for males and females.

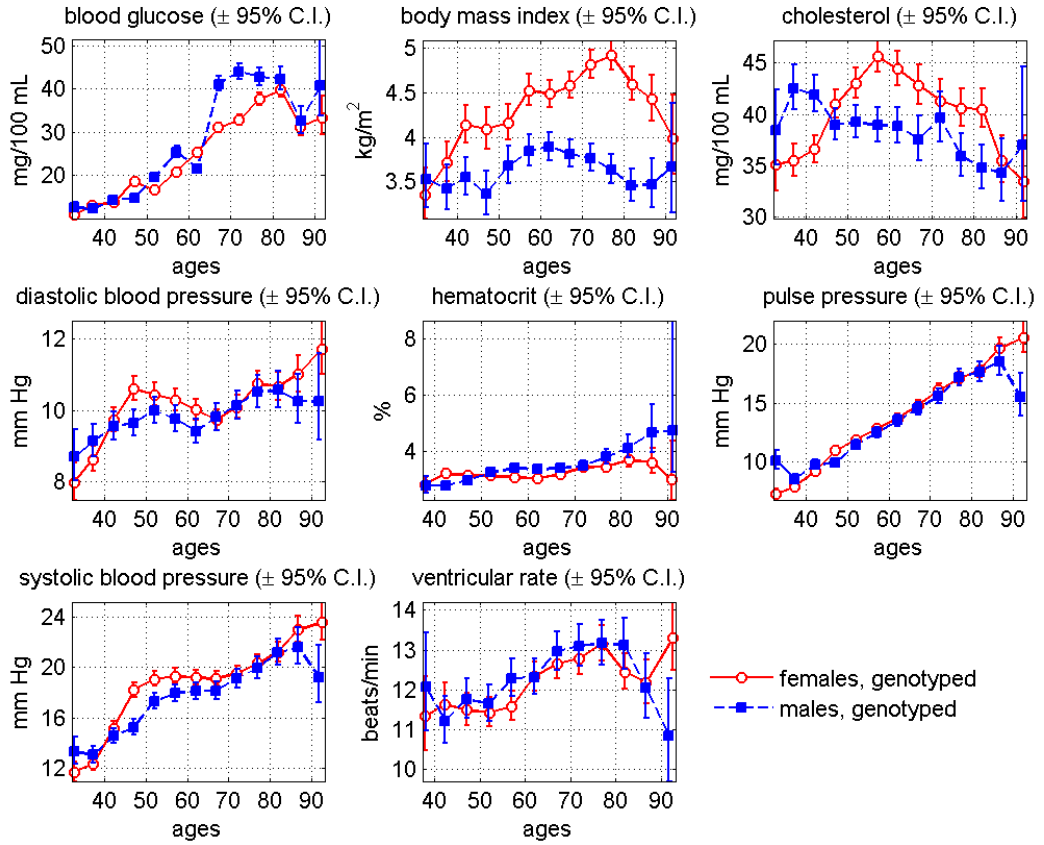

Figure 6. Age trajectories of standard deviations for eight physiological indices including blood glucose, body mass index, cholesterol, diastolic blood pressure, hematocrit, pulse pressure, systolic blood pressure and ventricular rate for genotyped males and females in the data from the original FHS cohort.

Genetic analyses of data on life span from the original FHS cohort

We combined data on 500,000 and 50,000 SNPs available in the Framingham data. This procedure resulted in 549,157 SNPs. We applied GWAS quality control (QC) procedures to the data on genotyped individuals from the original FHS cohort: sample call rate ≥95 percent; minor allele frequency (MAF) >5 percent; Hardy-Weinberg equilibrium (HWE) $>10^{-7}$. Altogether 1,111 individuals (432 males and 679 females) had a sample call rate ≥95 percent.

The influence of statistical models used in GWAS on the results of genetic analyses. In our recent analysis,[14] we showed that alleles selected in human longevity GWAS depend on a statistical model describing the connection between longevity traits and genetic variants. To reduce the effect of the model on the selection result, we used six statistical procedures for the alleles' selection and identified an overlapping set of 27 SNPs showing an effect on life span across all six statistical procedures. We found that the "longevity SNPs" were located in/near genes largely involved in cell proliferation and apoptosis/senescence pathways.

The influence of QC procedure used in GWAS on the results of genetic analyses. Figure 7 illustrates how different values of call rates used in the quality control procedure influence the number of detected genetic variants. One can conclude from this diagram that changing parameters in call rates influences the sample size as well as the number of detected genetic variants. In cases when sample size of the data is limited, the stringent

[14] Yashin et al., "Polygenic Effects," 381–94.

control for sample call rate may substantially reduce the power of genetic analyses. The difference in results may explain why it is difficult to replicate findings using independent populations. The difference in QC procedures used in different studies might make a substantial contribution to nonreplication.

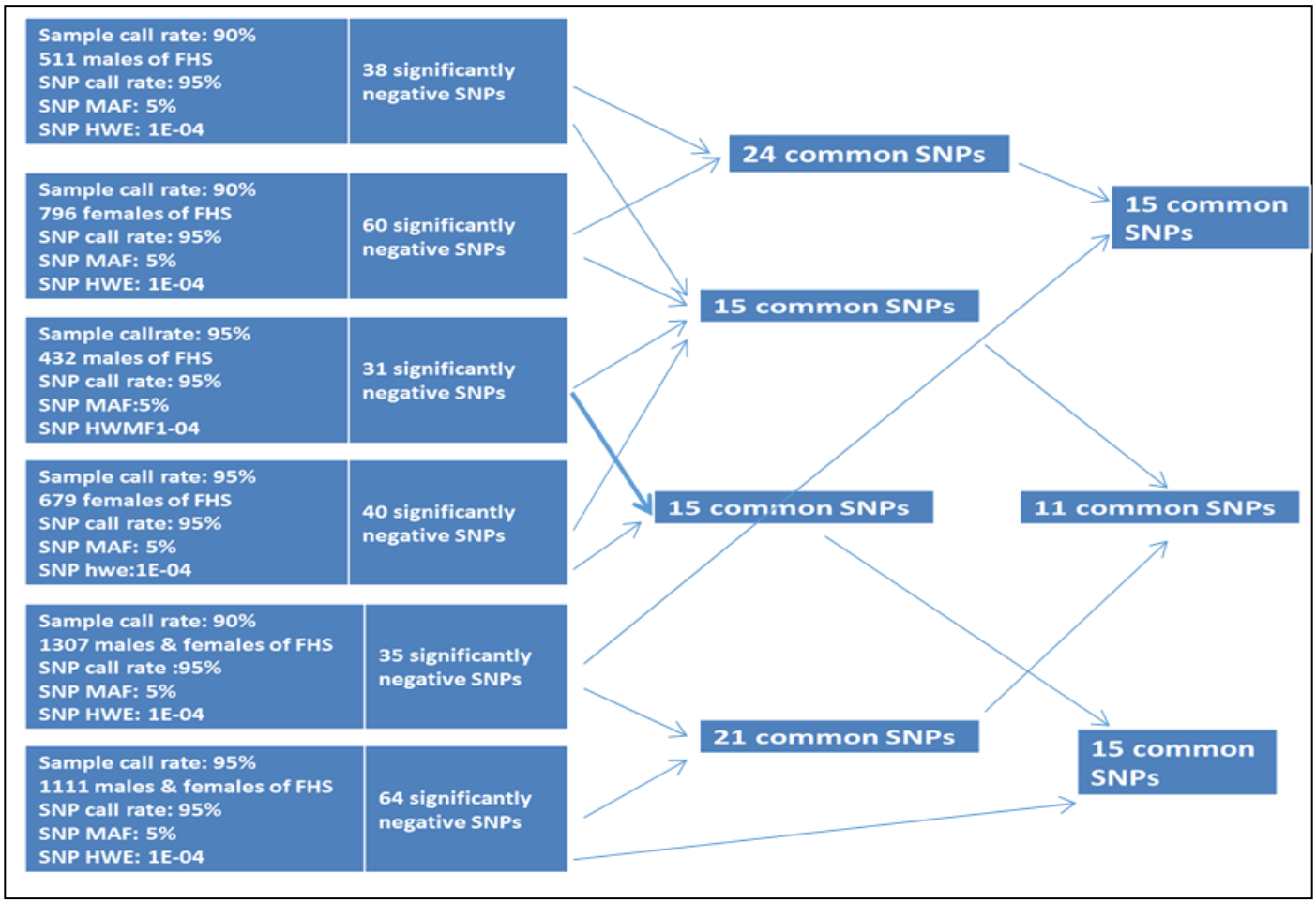

Figure 7. Effects of sample call rate on the number of genome-wide statistically significant genetic variants in GWAS of the original FHS cohort.

Longevity or vulnerability genes? It is important to note that a substantial portion of the genotyped individuals from the original FHS cohort has information on their life spans. Among 1,307 study participants, only 203 people had censored life spans. To use a mixed-effect model in the analyses of data on life span using EMMAX software,[15] we estimated residual life spans for individuals censored at a given age by calculating average life span of deceased study participants who survived up to this age. Then we used these estimates to impute life span for individuals with censored data. In the case of 95 percent sample call rates, the life spans of 203 individuals were imputed.

The GWAS of life span data (which used an additive genetic model) resulted in selection of 24 genome-wide significant genetic variants (minor alleles) common for males and females (table 2). Note that all these variants have negative associations with life span so they will be called "frailty" or "vulnerability" alleles. In these analyses, smoking and birth cohorts were used as observed covariates.

[15] Kang, Cho, and Zhao, "Practical Issues," 415–40.

Table 2. List of selected SNPs whose minor alleles have significant negative associations with life span. Data from the original FHS cohort. The columns in the table denote: (1) SNP number; (2) chromosome number; (3) number of minor alleles in a sample; (4) the total number of alleles in a sample; (5) minor allele frequencies in a sample; (6) minor allele frequencies in HapMap; (7) minor allele frequencies in the 1,000 Genome Project.

rs num	Chr	# MA	# A	MAF	MAF HP	MAF 1000
rs5491	19	224	2128	**0.10563**	0	0.075
rs356430	5	218	2020	**0.107921**	0	0.017
rs1399453	12	225	2052	**0.109649**	0	0.024
rs1440483	11	190	2064	**0.092054**	0	0.054
rs1794108	11	159	2104	**0.07557**	0	0
rs2353447	8	230	2092	**0.109943**	0	0.02
rs2586484	17	236	2074	**0.11379**	0.008	0.012
rs2838566	21	252	2098	**0.120114**	0	0.11
rs3738682	1	166	2026	**0.081935**	0.017	0.011
rs4565533	9	447	2088	**0.21408**	0.09	0.06
rs4904670	14	291	2120	**0.137264**	0	0.03
rs5743998	11	198	2044	**0.096869**	0	0.012
rs6007952	22	356	2058	**0.172983**	0.05	0.06
rs6090342	20	266	2060	**0.129126**	0	0.28
rs7894051	10	426	2136	**0.199438**	0.05	0.1
rs8081943	17	148	2176	**0.068015**	0	0.03
rs8135777	22	216	1996	**0.108216**	0	0.023
rs9896996	17	209	2082	**0.100384**	0.035	0.04
rs9925881	16	144	2068	**0.069632**	0	0.05
rs9928967	16	137	2140	**0.064019**	0	0.03
rs9971555	11	232	2092	**0.110899**	0	0.02
rs10845099	12	380	2072	**0.183398**	0.093	0.323
rs11536959	20	155	2132	**0.072702**	0	0.017
rs17067605	5	167	2046	**0.081623**	0	0.004

To understand whether genetic variants showing negative associations with life span are also associated with age trajectories of physiological indices, we constructed age trajectories of eight physiological indices for carriers and noncarriers of each of 24 minor alleles. Figures 8A and 8B show average age trajectories of physiological indices for male carriers and noncarriers of minor alleles of rs5491 and rs9925881 SNPs from the original FHS cohort. These two alleles were selected to illustrate the difference in their associations with age trajectories of physiological indices.

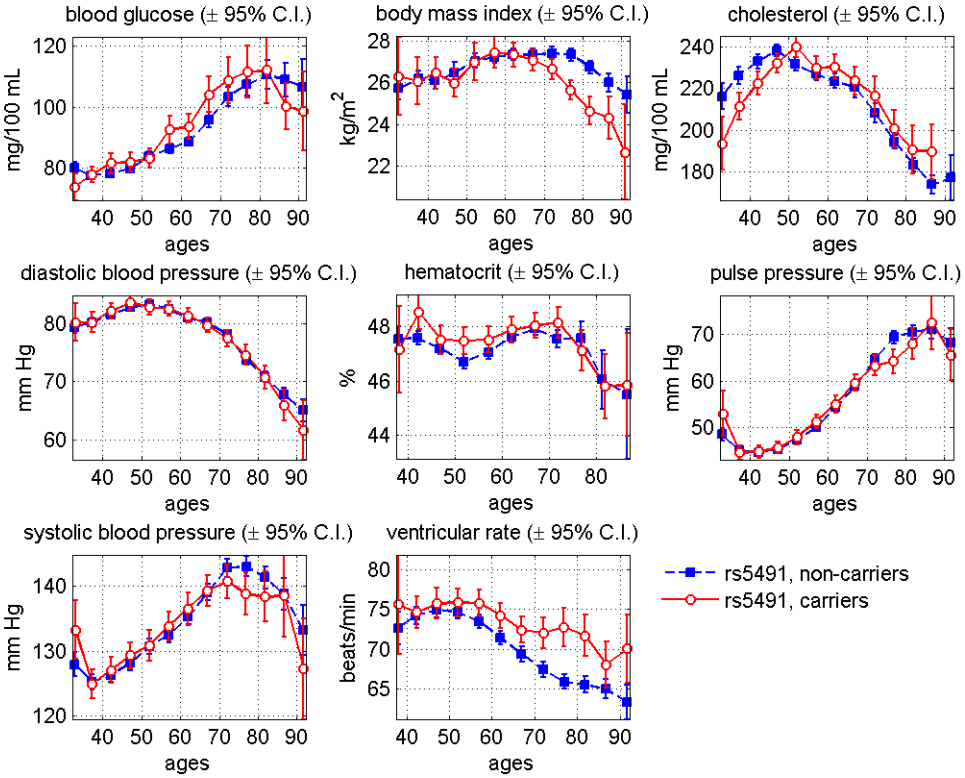

Figure 8A. Average age trajectories of physiological indices for male carriers and noncarriers of the minor allele of the rs5491 SNP from the original FHS cohort.

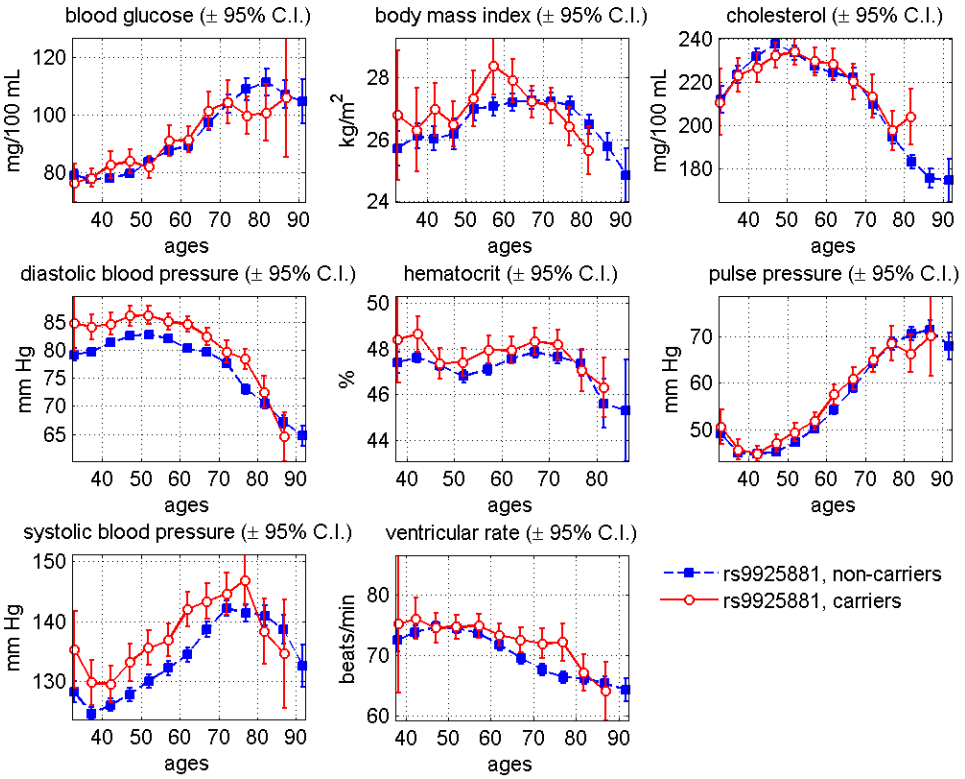

Figure 8B. Average age trajectories of eight physiological indices for male carriers and noncarriers of the minor allele of rs9925881 SNP from the original FHS cohort.

One can see from these figures that the main difference for carriers and noncarriers of the rs5491 SNP is in age trajectories of BMI and CH, and for the rs9925881 SNP, it is the average age trajectories of systolic and diastolic blood pressure.

The age structure of the population under study at the time of biospecimen collection provides one with additional information about the genetics of life span. This information can also be used for improving the accuracy of genetic analyses. The idea is based on the fact that participants of prospective studies usually have different ages at the time of biospecimen collection (see figure 9). To illustrate the benefits of the approach for joint analyses of the follow-up data and the data on ages at the time of biospecimen collection compared to the analyses of the follow-up data alone, we performed a simulation study assuming that carriers and noncarriers of some hypothetical allele in a population have the Cox's-type mortality rates $\mu(x \mid G) = \mu_0(x)e^{\gamma G}$, where $G = 0$ for noncarriers and $G = 1$ for carriers, and the baseline mortality $\mu_0(x)$ is the Gompertz function, i.e., $\ln \mu_0(x) = \ln a + bx$. In simulation, we used $\ln a = -9.0$ and $b = 0.08$, to produce reasonable survival patterns corresponding to human populations, and the proportion of carriers at birth, $p_0 = 0.25$. The parameter γ varied from −0.5 to 0.5 with the interval 0.1 to simulate scenarios with different effect sizes. For each set of model parameters defined above, we generated life spans of 4,500 individuals from the respective probability distributions, i.e., those corresponding to the hazard $\mu_0(x)e^{\gamma}$ for carriers and $\mu_0(x)$ for noncarriers. Then we assigned the hypothetical "ages at entry" into the study, which is also considered ages at the time of biospecimen collection, uniformly distributed over the interval between 40 and 100 years. Individuals with simulated life spans exceeding "age at entry" plus six years were considered censored at the "age at entry" plus six. Such design resembles the Long Life Family Study.[16] This procedure was repeated 1,000 times to generate 1,000 datasets (in each scenario with respective γ). We then estimated these data using the parts of the likelihood functions from a 2011 study[17] containing: (1) only follow-up information, and (2) follow-up information and information on ages at biospecimen collection. We calculated the power, i.e., the proportion of datasets for which the null hypothesis $\gamma = 0$ was rejected at the 0.05 level, in these two methods for different effect sizes, i.e., the values of the regression parameter γ. The results are shown in table 3.

Table 3. Power in simulation studies for two methods: (1) only follow-up information, and (2) follow-up information and information on ages at biospecimen collection. Power >0.8 is highlighted in ***bold italics***.

γ	RR	Follow-up only	Follow-up and ages
−0.5	0.607	***1.000***	***1.000***
−0.4	0.670	***0.997***	***1.000***
−0.3	0.741	***0.940***	***1.000***
−0.2	0.819	0.663	***0.938***
−0.1	0.905	0.198	0.435
0.0	1.000	0.058	0.043
0.1	1.105	0.223	0.464
0.2	1.221	0.666	***0.958***
0.3	1.350	***0.953***	***1.000***
0.4	1.492	***0.996***	***1.000***
0.5	1.649	***1.000***	***1.000***

[16] See description in Yashin et al., "Predicting Parental Longevity," 215–22.
[17] Arbeev et al., "Evaluation," 157–66.

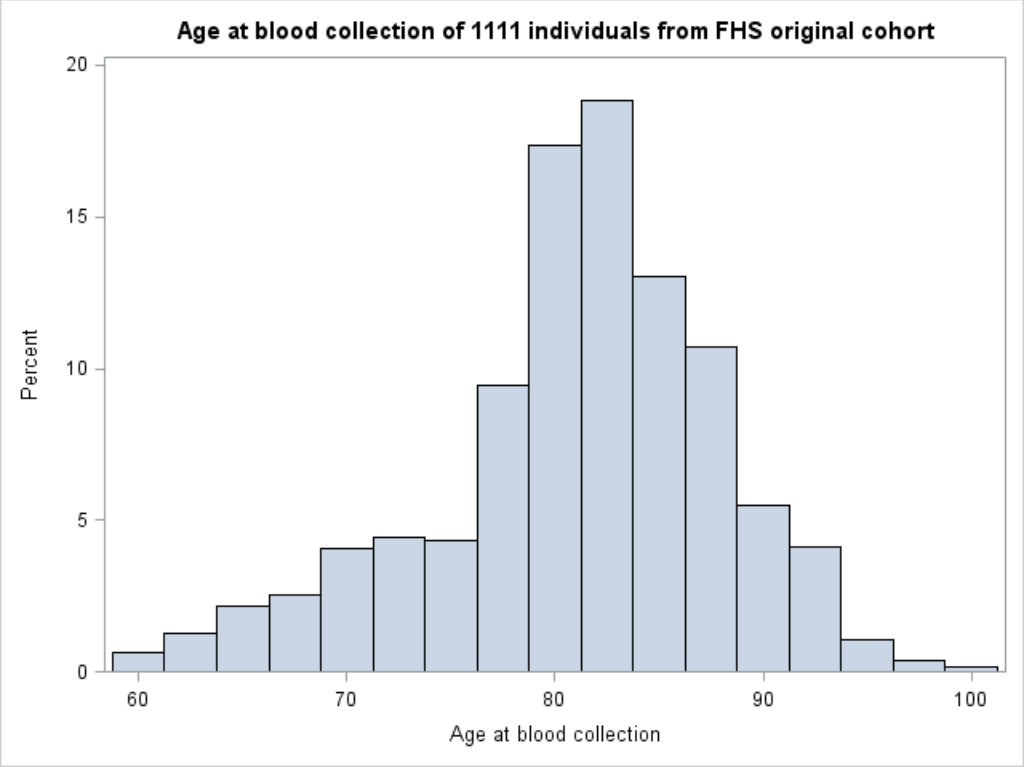

Figure 9. Age distribution of the members of the original FHS cohort at the time of biospecimen collection.

The results shown in table 3 indicate that the use of information on ages at biospecimen collection in addition to the follow-up data gives a substantial increase in power compared to the traditional approach that uses the follow-up data only. Note that these analyses can be implemented to the data on ages at disease onset instead on life span data. The effect, however, depends on duration of the follow-up period. In the case of a longer follow-up period, the relative contribution of the data on ages at the time of biospecimen collection to the improvement of the accuracy of statistical estimates will be smaller. Conversely, in the case of a shorter follow-up period, the data on ages at biospecimen collection play a more important role in differentiating the allele- or genotype-specific survival patterns from the data. The results of these analyses show that the approach described above may have important implications for GWA studies of human aging, health and longevity, especially in cases with short follow-up periods. The application of this approach to the analyses of data on 24 SNPs resulted in a substantial increase in the significance of detected p-values.

The genetic analyses of longitudinal data on aging, health and longevity using the genetic version of the stochastic process model, GenSPM

Analyses of longitudinal data on aging, health and longevity aimed at elucidating the effects of genetic factors on biological mechanisms require interdisciplinary efforts and integration of these efforts within a special methodological framework. The framework is needed to properly incorporate state-of-the-art concepts and the principles of systems biology that underlay aging-related changes in human organisms into statistical models, which then will be used in statistical analyses of data. Many statistical methods that involve joint modeling of longitudinal and time-to-event data have been developed during the last several decades using both frequentist and Bayesian approaches.

Although all these approaches are flexible enough and useful in many different applications, their role in analyses of aging, health and longevity is limited. The “universality” of these methods precludes addressing

specific biological problems clarifying biological mechanisms of aging, or incorporating the knowledge about regularities of aging-related changes accumulated in the field. State-of-the-art advances in systems biology of aging-related processes include the concepts of age-specific physiological norms,[18] allostasis and allostatic load,[19] the decline in adaptive capacity with age (homeostenosis),[20] the decline in stress resistance with age[21] and short-scale stochasticity,[22] e.g., stochasticity associated with erratic behavior of physiological parameters or dynamics of blood pressure at a time scale of minutes.

Another point to consider is the shape of the hazard rate as a function of physiological variables. Many epidemiological studies provide evidence of U- or J-shaped risks as functions of different physiological characteristics of health.[23] Therefore, the use of such quadratic (U- or J-shaped) hazards in the analyses is biologically meaningful. An important class of models for joint analyses of longitudinal and time-to-event data uses a stochastic process for description of longitudinal measurements and a quadratic hazard as a function of physiological variables. An initial version of such models was put forth in a 2007 report.[24] The model's various extensions have been formulated and applied in different contexts to investigate mechanisms of aging-related changes in connection with morbidity or mortality risks.[25] The advantage of this approach is that it allows for incorporating the concepts and mechanisms of aging-related changes mentioned above on the basis of the common framework provided by this model.

The version of SPM for analyses of genetic data was developed in a 2009 effort.[26] This GenSPM permits:

(1) joint analyses of genotyped and nongenotyped subsamples of longitudinal data to make use of all available information and to increase the accuracy/power of estimates compared to analyses of genotyped subsample alone;
(2) evaluation of indirect genetic effects, e.g., associated with unobservable or unmeasured risk factors, mediated by age trajectories of physiological variables collected in a longitudinal study; and
(3) incorporation of concepts and mechanisms of systems biology underlying aging-related changes in organisms that are not directly measured in longitudinal data but can be estimated from individual age trajectories of physiological indices and time-to-event data.

Specifically, this model permits evaluation of hidden (unobserved) biomarkers driving individual physiological change and affecting population characteristics. These include the aforementioned concepts of age-specific physiological norms, the allostatic load, homeostenosis, the decline in stress resistance with age, the short-scale stochasticity and respective hazard rates, for carriers and noncarriers of a selected allele/genotype. This model can be straightforwardly extended to incorporate "static" covariates to evaluate their modulating role in life

[18] Prospective Studies Collaboration, "Age-Specific Relevance," 1903–13; Palatini, "Need for a Revision," 622–25; and Westin and Heath, "Thresholds," 1461–62.

[19] Karlamangla, Singer, and Seeman. "Reduction in Allostatic Load," 500–07; and Seeman et al., "Allostatic Load," 4770–75.

[20] Lund et al., "Transcriptional Profile," 1566–73; and Troncale, "The Aging Process," 111–114, 120–22.

[21] Yashin et al., "Stochastic Model," 538–51; Hall et al., "Aging Reduces," 749–59; and Ukraintseva and Yashin, "Individual Aging," 163–96.

[22] Goldberger, Peng, and Lipsitz, "What is Physiologic Complexity," 23–26.

[23] Allison et al., "Hypothesis Concerning the U-Shaped Relation," 339–49; Boutitie et al., "J-Shaped Relationship," 438–48; Kulminski et al., "Body Mass Index," 105–10; Kuzuya et al., "J-Shaped Relationship," 367–68; Mazza et al., "Serum Uric Acid," 99–105; Okumiya et al., "U-Shaped Association," 1415–21; Protogerou et al., "Diastolic Blood Pressure," 172–80; Troiano et al., "Relationship Between Body Weight," 63–75; and Witteman et al., "J-Shaped Relation," 504–07.

[24] Yashin et al., "Stochastic Model," 538–51.

[25] Arbeev et al., "Evaluation," 157–66; Yashin et al., "Model of Hidden Heterogeneity," 1–10; Yashin et al., "What Age Trajectories," 191–200; Yashin et al., "Maintaining Physiological State," 611–18; Yashin et al., "Exceptional Survivors," 257–65; Yashin et al., "New Approach," 5336–50; Yashin et al., "Quadratic Hazard Model," 177–88; Yashin et al., "How Lifespan Associated Genes"; Arbeev et al., "Genetic Model," 103–11; Arbeev et al., "Effect of the APOE Polymorphism"; Yashin et al., "Patterns of Aging Related Changes," Society of Actuaries; Akushevich et al., "New Stochastic Carcinogenesis Model," 16–30; and Tolley, "Using the Random Walk Model," 191–92.

[26] Arbeev et al., "Genetic Model," 103–11.

course genetic effects.

The GenSPM[27] was applied to the analyses of longitudinal data on serum cholesterol and diastolic blood pressure for *APOE* and non-*APOE* subsamples of the original FHS cohort. The details of model construction and the likelihood maximization procedure are described in the report.[28] Here we give only a brief description of the model to help understand the research results.

The evolution of physiological variables Y_t over age t is described by the stochastic differential equation

$$dY_t = a(t,G)(Y_t - f_1(t,G))dt + B(t,G)dW_t, \quad (1)$$

with the initial condition $Y_{t_0} \sim N(f_1(t_0,G),\sigma_{0G})$. Here G (G = 0, 1; $P(G=1)=p_1$) is a discrete random variable characterizing the absence (G = 1) or presence (G = 0) of the *APOE* e4 allele in a person's genome, W_t is a Wiener process independent of Y_{t_0} and G. The coefficient $B(t,G)$ was considered constant ($B(t,G)=\sigma_{1G}$) in these applications.

The effect of allostatic adaptation $f_1(t,G)$[29] is described as quadratic function of t: $f_1(t,G)=a_{f_1}^G+b_{f_1}^G t+c_{f_1}^G t^2$. This choice comes from the empirical observations of the average trajectories of the physiological variables in the FHS, which generally have a quadratic form, although, of course, these average trajectories do not necessary have to follow $f_1(t,G)$.

The negative feedback coefficient $a(t,G)$ is characterized by strength of homeostatic forces. The decline in the absolute value of this coefficient with age represents the decline in the adaptive (homeostatic) capacity with age ("homeostenosis"), which has been shown to be an important characteristic of aging.[30] We used a linear approximation of this coefficient as a function of age: $a(t,G)=a_Y^G+b_Y^G t$ (with $a_Y<0$ and $b_Y \geq 0$).

The U- or J-shapes of the mortality and morbidity risks as functions of various physiological variables and other risk factors were confirmed in a number of studies.[31] This indicates that a quadratic function can capture dependence of the risk on deviations of trajectories of a physiological variable Y_t from its "optimal" values.[32] Such function has been used to describe mortality rate conditional on Y_t and G:

$$\mu(t,Y_t,G)=\mu_0(t,G)+(Y_t-f_0(t,G))^2\mu_1(t,G). \quad (2)$$

Here $\mu_0(t,G)$ is the baseline hazard, $f_0(t,G)$ are "optimal" trajectories ("physiological norms"). We used the gamma-Gompertz (logistic) baseline hazards $\mu_0(t,G)$: $\mu_0(t,G)=\mu_0^0(t,G)\Big/(1+\sigma_{2G}^2\int_0^t\mu_0^0(u,G)du)$, where

[27] Ibid.

[28] Ibid.

[29] Yashin et al., "Stochastic Model," 538–51; and Arbeev et al., "Genetic Model," 103–11.

[30] Lund et al., "Transcriptional Profile," 1566–73; Troncale, "The Aging Process," 111–114, 120–22; Hall et al., "Aging Reduces," 749–59; and Rankin and Kushner, "Adaptive β-cell Proliferation," 1365–72.

[31] Allison et al., "Hypothesis Concerning the U-Shaped Relation," 339–49; Boutitie et al., "J-Shaped Relationship," 438–48; Kuzuya et al., "J-Shaped Relationship," 367–68; Okumiya et al., "U-Shaped Association," 1415–21; Protogerou et al., "Diastolic Blood Pressure," 172–80; and van Uffelen et al., "What is a Healthy Body Mass Index," 844–50.

[32] Yashin et al., "What Age Trajectories," 191–200; Yashin et al., "Health Decline," 291–302; Yashin et al., "Maintaining Physiological State," 611–18; Yashin et al., "Exceptional Survivors," 257–65; Arbeev et al., "Genetic Model," 103–11; and Arbeev et al., "Age Trajectories," 93–102.

$\mu_0^0(t,G) = a_{\mu_0}^G e^{b_{\mu_0}^G t}$.[33]

The coefficient $\mu_1(t,G)$ characterizes stress resistance. Its increase with age corresponds to the decline in stress resistance because it narrows U-shape of the risk, i.e., making an organism more vulnerable to deviations from the "optimal" values, which can be considered as a manifestation of the senescence process.[34] In our analyses, $\mu_1(t,G)$ was approximated by a linear function of age: $\mu_1(t,G) = a_{\mu_1}^G + b_{\mu_1}^G t$.

The average age trajectories of respective physiological variables in long-lived (life span ≥90 for females, ≥85 for males) carriers and noncarriers of the *APOE* e4 allele were considered as "optimal" trajectories $f_0(t,G)$ in the model.

The model specification allows for testing the hypotheses on the differences in aging-related characteristics, e.g., adaptive capacity and mean allostatic trajectories, between carriers and noncarriers of the e4 allele, on the decline in adaptive capacity with age, etc., using the likelihood ratio test. The likelihood optimization and the statistical tests have been performed using the optimization and statistical toolboxes in MATLAB.

The results of genetic analyses using GenSPM

Table 4 shows estimates of parameters of the baseline hazard ($\mu_0(t,G)$), the multiplier in the quadratic part of the hazard ($\mu_1(t,G)$), the adaptive capacity $a(t,G)$, the mean allostatic trajectory ($f_1(t,G)$) and other parameters of the GenSPM applied to the data on mortality and longitudinal measurements of CH and DBP from the original FHS cohort. The table also shows the results of testing null hypotheses about coincidence of various components of the model, such as adaptive capacity and mean allostatic trajectory, in carriers and noncarriers of the e4 allele and other hypotheses on dynamic characteristics of the components of the model in the genetic groups (see note after the table). Figures 11 and 12 show estimated components of the model such as the logarithm of the baseline hazard, the multiplier in the quadratic part of the hazard, the adaptive capacity coefficient and the mean allostatic trajectory for carriers and noncarriers of the *APOE* e4 allele evaluated from data on CH and DBP for males and females combined.

[33] Vaupel et al., "Biodemographic Trajectories," 855–60.
[34] Semenchenko et al., "Stress Resistance," 17–30; and Robb, Page, and Stuart, "Mitochondria," 12–27.

Table 4. Estimates of parameters of the genetic stochastic process model applied to data on mortality and longitudinal measurements of total cholesterol and diastolic blood pressure in female and male carriers (e4) and noncarriers (no e4) of the *APOE* e4 allele in the original Framingham Heart Study cohort)

Variable	Allele	Baseline hazard ($\mu_0(t,G)$)			Multiplier in quadr. part of hazard ($\mu_1(t,G)$)		Adaptive capacity ($a(t,G)$)		Mean allostatic trajectory ($f_1(t,G)$)			Other parameters		
		$\ln a_{\mu_0}^G$	$b_{\mu_0}^G$	σ_2^G	$a_{\mu_1}^G$	$b_{\mu_1}^G$	a_Y^G	b_Y^G	$a_{f_1}^G$	$b_{f_1}^G$	$c_{f_1}^G$	σ_0^G	σ_1^G	p_1
CH	e4	−5.05#	0.042	0.01	−0.0079	0.0021	−0.165†	1.993†	258.77†	1.016	−0.0556	51.11	24.18	0.302
	no e4	−5.65	0.052	0.00	−0.0105	0.0026	−0.072	1.161	223.51	1.141	−0.0492	38.34	14.11	
DBP	e4	−5.41†	0.067	0.00	−0.1297#	0.0371	−0.153	0.000	94.85†	−0.073	−0.0107	13.92	6.97	0.300
	no e4	−6.32	0.082	0.00	−0.1783†	0.0018	−0.150	0.000	80.52	0.199	−0.0115	9.16	5.07	

Notes:

1) The estimates of some parameters are rescaled for better visibility in the table: $a_{\mu_1}^G$ are multiplied by 10^4; $b_{\mu_1}^G$ are multiplied by 10^5; b_Y^G are multiplied by 10^3.
2) The symbols after the numbers in the following column denote p-values (evaluated by the likelihood ratio test) for different null hypotheses:
   Column $\ln a_{\mu_0}^G$: baseline hazard rates coincide in carriers and noncarriers of the e4 allele, i.e., $\mu_0(t,\mathrm{no}\,e4)=\mu_0(t,e4)$ (respective symbols are shown in rows no e4)
   Column $a_{\mu_1}^G$: zero quadratic part of the hazard (separately for carriers and noncarriers), i.e., $\mu_1(t,\mathrm{no}\,e4)=0$ for rows no e4, $\mu_1(t,e4)=0$ for rows e4
   Column $b_{\mu_1}^G$: age-independent U-shapes of the hazard (separately for carriers and noncarriers), i.e., $b_{\mu_1}^1=0$ for rows no e4, $b_{\mu_1}^0=0$ for rows e4
   Column a_Y^G : adaptive capacities coincide in carriers and noncarriers, i.e., $a(t,\mathrm{no}\,e4)=a(t,e4)$ (respective symbols are shown in rows no e4)
   Column b_Y^G : no aging-related decline in the adaptive capacity (separately for carriers and noncarriers), $b_Y^1=0$ for rows no e4, $b_Y^0=0$ for rows e4
   Column $a_{f_1}^G$: "mean allostatic trajectories" coincide in carriers and noncarriers, i.e., $f_1(t,\mathrm{no}\,e4)=f_1(t,e4)$ (respective symbols are shown in rows no e4)

   The symbols in these columns denote: †: p < 0.0001; §: 0.0001 ≤ p <0.001; #: 0.001 ≤ p <0.01; *: 0.01 ≤ p <0.05, for respective null hypotheses. The absence of symbols after the numbers in these columns means that respective p-values exceed 0.05. Note that all other columns in the table, except the columns mentioned above, are not used to represent information on testing any null hypotheses and therefore they do not contain any symbols.

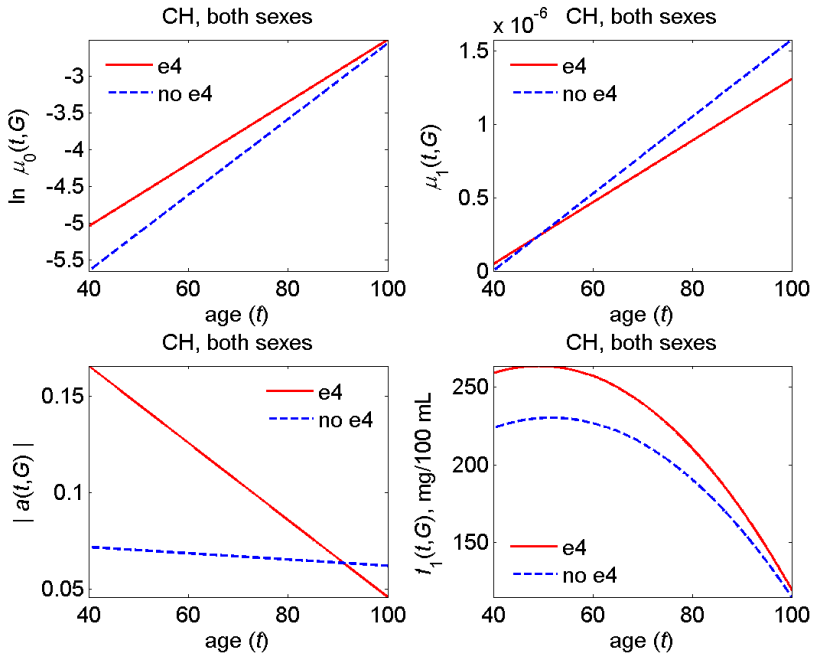

Figure 11. Application of the genetic stochastic process model to longitudinal measurements of total cholesterol and data on mortality in the Framingham Heart Study original cohort: Estimates of the logarithm of the baseline hazard ($\ln \mu_0(t,G)$, top left panel), the multiplier in the quadratic part of the hazard ($\mu_1(t,G)$, top right panel), the adaptive capacity (the absolute value of the feedback coefficient, $|a(t,G)|$, bottom left panel) and the mean allostatic trajectory ($f_1(t,G)$, bottom right panel) for carriers (e4) and noncarriers (no e4) of the *APOE* e4 allele

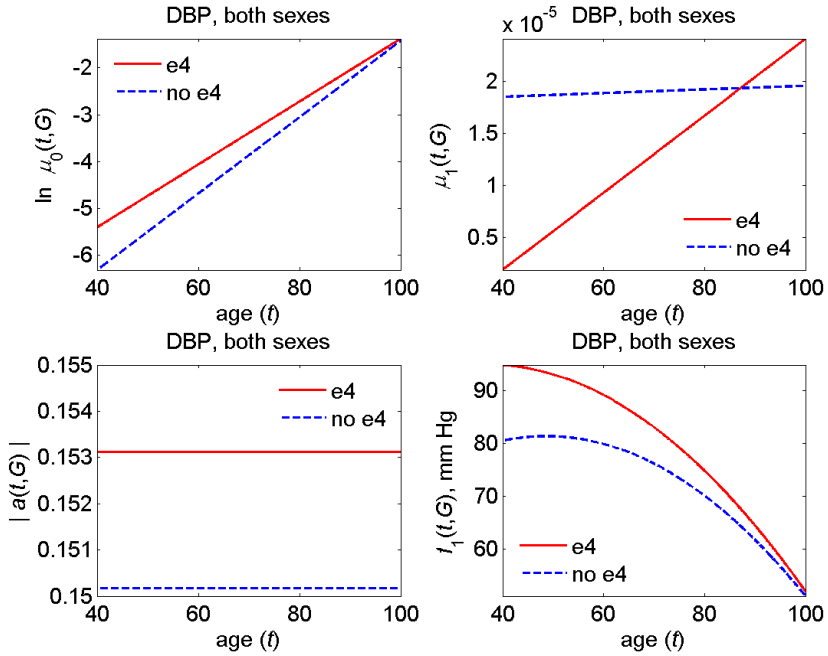

Figure 12. Application of the genetic stochastic process model to longitudinal measurements of diastolic blood pressure and data on mortality in the Framingham Heart Study original cohort: Estimates of the logarithm of the baseline hazard ($\ln \mu_0(t,G)$, top left panel), the multiplier in the quadratic part of the hazard ($\mu_1(t,G)$, top right panel), the adaptive capacity (the absolute value of the feedback coefficient, $|a(t,G)|$, bottom left panel) and the mean allostatic trajectory ($f_1(t,G)$, bottom right panel) for carriers (e4) and noncarriers (no e4) of the *APOE* e4 allele.

One can see from table 4 that the null hypotheses on the equality of baseline hazard rates in carriers and noncarriers of the e4 allele (column $\ln a^G_{\mu_0}$) are rejected for both physiological variables. Figures 11 and 12, top left panels, illustrate the patterns of the logarithm of baseline hazard rates estimated for both physiological variables and both sexes. They show that noncarriers of the e4 allele have lower baseline rates at younger ages, i.e., smaller $\ln a^G_{\mu_0}$, but they increase faster, i.e., they have larger $b^G_{\mu_0}$, than the rates for carriers of the e4 allele, resulting in the intersection of the rates at the oldest ages (around 100 years). This observation is in line with the findings in the literature that the effect of the e4 allele on survival diminishes with age[35] and that there is a lack of association of *APOE* alleles with survival of centenarians.[36] The table also shows that all parameters differ among carriers and noncarriers of the e4; however, some differences are not statistically significant.

The null hypotheses on the zero quadratic part of the hazard (column $a^G_{\mu_1}$ in table 4) are rejected in all cases for DBP but not for CH. This suggests that deviations of DBP from the "optimal" trajectories results in a more substantial increase in the risk of death than in the case of CH. Figure 11, top right panel, shows the tendency of increasing in $\mu_1(t,G)$ for CH; however, this increase is not statistically significant. Figure 12, top right panel, shows faster increases in $\mu_1(t,G)$ for e4 carriers in the case of DBP. This corresponds to the narrowing of the U-shape of corresponding mortality risk (as a function of DBP) with age. Hence the "price" for the same magnitude of deviation from "optimal" values of DBP (in terms of an absolute increase in the mortality risk compared to the baseline level at that age) becomes higher for carriers than for noncarriers at older ages. This can be considered as a manifestation of the decline in resistance to stresses with age,[37] which is an important characteristic of the aging process[38] contributing to the development of aging-related diseases and death. Note that e4 noncarriers have a narrower U shape for ages up to 85. It is important to note that our approach allows for indirect evaluation of this characteristic for carriers and noncarriers of the e4 allele in the absence of specific information on external disturbances (stresses) affecting individuals during their life course (such data are not available in the FHS).

The analyses also revealed different age dynamics of the adaptive capacity in carriers and noncarriers of the e4 allele for different physiological variables. The null hypotheses on the equality of the adaptive capacity in carriers and noncarriers (column a^G_Y in table 4) are rejected in case of CH. Figures 11 and 12, bottom left panels, show that carriers of the e4 allele have better adaptive capacities than noncarriers of this allele. The age dynamics of the adaptive capacity are different for CH and DBP. These observations indicate that the mechanisms underlying the decline in the adaptive capacity in carriers and noncarriers of e4 may not work universally for all physiological indices. The decline in adaptive capacity is an important feature of aging[39] that may contribute to development of aging-related diseases and death. However, direct measurements of the adaptive capacity are typically lacking in available longitudinal studies of aging, health and longevity. The use of the feedback coefficient in the equation for the age dynamics of a physiological variable in our model allows us to indirectly evaluate this from the data because the absolute value of this feedback coefficient characterizes the adaptive capacity.[40]

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[35] Ewbank, "Mortality Differences," 146–55.

[36] Louhija et al., "Survival in Finnish Centenarians," 1007–08.

[37] Yashin et al., "Stochastic Model," 538–51; and Arbeev et al., "Age Trajectories," 93–102.

[38] Semenchenko et al., "Stress Resistance," 17–30; and Robb, Page, and Stuart, "Mitochondria," 12–27.

[39] Lund et al., "Transcriptional Profile," 1566–73; Troncale, "The Aging Process," 111–114, 120–22; Hall et al., "Aging Reduces," 749–59; and Rankin and Kushner, "Adaptive β-cell Proliferation," 1365–72.

[40] Arbeev et al., "Evaluation," 157–66; Yashin et al., "Stochastic Model," 538–51; Yashin et al., "Quadratic Hazard Model," 177–88; and Arbeev et al., "Genetic Model," 103–11.

The null hypothesis on the equality of the mean allostatic trajectories in carriers and noncarriers (column $a_{f_1}^G$ in table 4) are rejected for both CH and DBP. This indicates that the processes regulating the age dynamics of physiological variables in carriers and noncarriers of the e4 allele force their age trajectories to follow different curves (which also do not coincide with the "optimal" trajectories). Figures 11 and 12, bottom right panels, show that age trajectories of both CH and DBP in e4 carriers are forced to larger values compared to noncarriers of the allele, although the difference between carriers and noncarriers diminishes at the oldest ages.

Discussion

Advances in genotyping technology provided researchers with a large amount of data on the genetic backgrounds of a large number of individuals for whom phenotypic longitudinal data were collected. This information stimulated numerous genome-wide association studies of complex traits with a hope of detecting genetic mechanisms involved in regulation of these traits. However, despite the fact that many genetic variants showed their association with complex traits, they explain only a small portion of genetic variability in these traits, indicating that many more influential genetic factors and regulatory mechanisms still have to be identified.[41] Testing associations for many genetic variants in GWAS requires correction for multiple comparison.[42] As a result, many genetic variants that showed their associations with such traits have not reached a genome-wide level of statistical significance. The results of genetic analyses of aging- and longevity-related traits produced additional challenges for the researchers. Most genetic variants detected in earlier analyses of such traits failed to be replicated in studies of the same traits in independent populations.[43] The results of GWAS of human aging and life span are sensitive to the type of statistical model used in the allele (genotype) selection procedure,[44] indicating that conclusions from such analyses have to be used with care and mechanisms of genetic influence on aging and life span require more accurate descriptions. The results are also sensitive to the set of rules used in the quality control procedures, which specify sets of genetic factors and fix the sample of study subjects appropriate for genetic analyses. The fact that the researchers performing such procedures often use different sets of rules casts doubts on the legitimacy of comparison of the results of different studies, as well as on the results of meta-analyses of the results of studies in which different QC procedures have been used. Another problem in genetic studies of aging and longevity is that dealing with linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) in these studies follows standards used in the analyses of other complex traits. However, this strategy is erroneous because it ignores the fact that mortality selection in a genetically heterogeneous population affects LD and HWE parameters in population cohorts.

The process of mortality selection in which the "longevity" or "vulnerability" alleles or genotypes are involved can also modify genetic structure in the populations of the old and oldest-old individuals compared to the younger groups of individuals. This property indicates that controlling for possible population stratification,

[41] Manolio et al., "Finding the Missing Heritability," 747–53; Makowsky et al., "Beyond Missing Heritability," e1002051; Eichler et al., "Missing Heritability," 446–50; Zuk et al., "Mystery of Missing Heritability," 1193–98; and Marian, "Elements of 'Missing Heritability,' " 197–201.

[42] Nyholt, "Simple Correction," 765–69; Gao et al., "Avoiding the High Bonferroni Penalty," 100–05; and Li et al., "Evaluating the Effective Numbers," 747–56.

[43] Lunetta et al., "Genetic Correlates," S13; Newman et al., "Meta-Analysis," 478–87; Nebel et al., "Genome-Wide Association Study," 324–30; Walter et al., "Genome-Wide Association Study," 2109.e15–e28; Barzilai et al., "Place of Genetics," 589–94.

[44] Yashin et al., "Polygenic Effects," 381–94.

e.g., due to the differences in ancestry,[45] has to be done with care. Without such care controlling for population, stratification can substantially reduce the estimates of associations of genetic variants with longevity traits.

It is also unclear how to deal with the multifactorial nature of the traits in GWAS, when the same value of the trait can be reached for different combinations of genetic variants, as well as in combinations of genetic variants and nongenetic factors. It remains unclear how genetic influence on aging and life span (individual or collective) is mediated by physiological variables, how these variables change during the life course of individuals with different genetic background, how they influence age patterns of incidence rates of aging-related chronic diseases and corresponding cause-specific mortality rates, and how associations of genetic variants with longevity-related traits change with increasing age. It is also unclear which combinations of genetic variants make most significant associations with exceptional longevity, and which age patterns of aging-related changes in biomarkers correspond to such combinations.

We use the genetic SNP data that passed appropriate quality control procedures, the data on the apolipoprotein E (*APOE*) common polymorphisms (e2, e3 and e4) and phenotypic longitudinal data collected in the FHS (with more than 60 years of follow up for the participants of the original FHS cohort) to investigate problems described above.[46] The genetic analyses of life span data are performed using several methods of GWAS that include advanced methods of joint analyses of data from different datasets. These methods explore several specifications of longevity phenotypes, implement control for family structure and population stratification, and take incompleteness of demographic data on life span (including left truncation and right censoring) into account. The methods allow for evaluating pleiotropic effects of selected genetic variants on aging, health and longevity traits. We also showed how genetic influence on hidden biomarkers of aging can be investigated using a stochastic process model of human mortality and aging. We also investigated the role of alleles of the *APOE* polymorphism in risks of major human diseases including cardiovascular disease, cancer and neurodegenerative diseases.

Our results show the complex role of the *APOE* gene in risks of the selected diseases, which are age, generation and gender specific. The results highlight antagonistic effects at different ages, across generations and across diseases. The analyses show that new methods of GWAS of human longevity provide researchers with efficient tools for genetic analyses of complex traits. Some of these variants change their associations with life span during the life course. Variants associated with human longevity are also associated with aging-related changes in physiological indices, as well as with mortality rates by cause. A number of detected variants show pleiotropic effects on disease traits and dynamic characteristics of aging-related changes developing during the life course.

The application of the GenSPM revealed different patterns of regularities in aging-related characteristics (adaptive capacity, decline in stress resistance, mean allostatic trajectories and the baseline hazard rate) in carriers and noncarriers of the *APOE* e4 allele. Such aging-related characteristics cannot be calculated directly from the longitudinal data because of the lack of respective measurements.

These differential patterns of aging-related characteristics may contribute to differences between the shapes of survival functions and average age trajectories of respective physiological variables in carriers and noncarriers of the e4 allele as well as between females and males. The underlying determinants of such differences in aging-related characteristics require additional studies. The analyses confirmed that genetic

[45] Price et al., "Principal Components Analysis," 904–09; Ma and Amos, "Theoretical Formulation," e12510; and Yang et al., "Genomic Inflation Factors," 807–12.

[46] Dawber, *The Framingham Study.*

influences on life span are realized through dynamic mechanisms regulating changes in physiological variables during the life course. The average aging-related changes in the eight selected physiological variables are likely to be driven by hidden components of aging changes and by genetic factors. The ability of advanced methods of statistical modeling to estimate hidden components of aging changes in humans indicates that the approach can be further extended to perform more comprehensive analyses of available data by incorporating relevant biological knowledge about aging into statistical models. The use of such models in statistical analyses of data will help researchers untangle complex age-dependent dynamic relationships among biomarkers and elucidate roles of genes and nongenetic factors in aging, health and life span.

Acknowledgements. The FHS project is conducted and supported by the National Heart, Lung and Blood Institute (NHLBI) in collaboration with Boston University (N01 HC25195). The FHS data used for the analyses were obtained through the database of Genotypes and Phenotypes (dbGaP). The authors acknowledge the investigators that contributed the phenotype and genotype data for this study. This manuscript was not prepared in collaboration with investigators of the FHS and does not necessarily reflect the opinions or views of the FHS, Boston University or the NHLBI. This work was partly supported by National Institutes of Health/National Institute of Aging (NIH/NIA) grants R01AG030612, R01AG030198, R01AG032319 and 1R01AG46860-01.

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