

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 Genomic Epidemiology Consortium

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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 or older ($n = 1,836$); the comparison group comprised cohort members who died between the ages of 55 and 80 years ($n = 1,955$). In a second discovery stage, additional genotyping was conducted in the Leiden Longevity Study cohort and the Danish 1905 cohort.

Results. There were 273 single-nucleotide polymorphism (SNP) associations with $p < .0001$, but none reached the prespecified significance level of 5×10^{-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 6.77×10^{-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 (odds ratio = 0.82). Associations of interest in a homologue of the longevity assurance gene (*LASS3*) and *PAPPA2* were not strengthened in the second stage.

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

Key Words: Longevity—Genome-wide association study—Meta-analysis.

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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 or insulin-like growth factor and insulin (GH, IGF, INS) axis—may play key roles (9–12). Model organisms suggest that inhibiting the GH, IGF, or 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 prolongevity transcription pathways. Clinical and epidemiological investigations, including candidate gene studies, have suggested that inflammation pathways may affect life span 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 (FHS) (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. Furthermore, we identified two independent cohorts of long-lived individuals, the Leiden Longevity Study cohort and the Danish 1905 cohort, to evaluate initial findings for the strongest allelic associations for longevity in a second discovery stage.

METHODS

Cohorts for Heart and Aging Research in Genomic Epidemiology 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 (AGES-Reykjavik) (31), the Cardiovascular Health Study (CHS) (32), FHS (33–36), and the Rotterdam Study (RS) (37).

The AGES-Reykjavik was funded by the National Institute on Aging (NIA) 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 was conducted in 5,764 men and women enrolled in 2002–2006 who were sampled from the 11,549 survivors of the AGES-Reykjavik of 30,000 men and women sampled from the 1907–1935 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 ($n = 5,201$) were recruited in 1989–1990, with an additional 687 minorities recruited in 1992–1993. The FHS is an NHLBI contract-funded cohort study initiated in 1948 to study determinants of CVD and other major illnesses. The original cohort comprised 5,209 men and women aged 28–62 years at enrollment who have undergone routine biennial examinations (33,34). In 1971, 5,124 offspring of the original cohort participants and offspring spouses, aged 5–70 years, were enrolled into the Framingham Offspring Study. Offspring participants have been examined approximately every 4–8 years (35,36). In the 1990s, DNA was obtained for genetic studies from surviving original cohort and offspring participants. The RS was planned and designed in the early 1990s as a longitudinal study investigating the incidence and progression of diseases in the elderly participants. From 1991 to 1995, all inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were aged 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 participants 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 1,836 persons who achieved longevity (144 from AGES-Reykjavik, 557 from CHS, 362 from FHS, and 773 from the RS), and the comparison group had 1,955 participants (122, 544, 355, and 934 participants from the AGES-Reykjavik, CHS, FHS, and RS, respectively). To facilitate comparison of results across studies, we imputed to 2.5 million SNPs using the HapMap Centre d'Etude du Polymorphisme Humain European Ancestry-genotyped samples as a reference. The effective sample size for all but one of the top SNPs was more than 80% of the full sample size of 3,791, indicating that the SNPs that were not 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 four-study meta-analysis ($p < 10^{-4}$), we selected the 22 SNPs that had been tested in all four CHARGE cohorts in two additional Caucasian cohorts: the Leiden Longevity Study cohort and the Danish 1905 cohort. We excluded the two SNPs that could not be genotyped or imputed in all four 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 89–104 years), 1,750 offspring (mean age 61 years, range 39–81 years), and 758 partners of offspring (mean age 60 years, range 36–79 years) were included. The additional genotyping of selected SNPs was undertaken in all 950 long-lived probands, and these were compared with the 744 partners of their offspring and an additional 680 blood bank donors

(60% men, mean age 31 years, range 18–40 years). All 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 92–93 years (41). Of the 3,600 participants 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 biologic material. The current genetic study comprises a total of 1,644 participants from this survey, mean age 93 years (range 92–93 years), 28% men. A comparison group included 2,007 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) (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 two SNPs that did not pass quality control and two 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 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 greater than 0.01 and were genotyped or imputed in all studies with variance ratio greater than 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 *df*. 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 90 years or older versus dying between ages 55 and 80 years 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 earlier. Finally, using the top 24 results, we conducted a pathway analysis with the Database for Annotation, Visualization and Integrated Discovery (<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 10–20 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. Whereas 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 less than 1.05 (45). After meta-analysis, overall inflation of the meta-analysis *p* values was minor ($\lambda_{GC} = 1.034$; Figure 1). None of the SNP–longevity associations achieved the prespecified 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 $p < 10^{-5}$ (Supplementary Table 2). Under the null hypothesis that there are no associations in the genome, we would expect $0.0001 \times \sim 2.3$ million = ~ 230 hits. Table 2 shows the top 24 independent SNPs associated with longevity along with the number of supporting SNPs (additional SNPs with linkage disequilibrium $r^2 > .80$ and $p < 10^{-4}$). Thus, for example, there were

Table 1. Characteristics of Longevity Cases and Comparison Group at DNA Collection

Characteristic, <i>M (SD)</i> or %	CHS		Framingham Heart Study		Rotterdam Study		AGES-Reykjavik	
	Survival Age to >90 y, <i>n</i> = 557	Comparison Group, <i>n</i> = 544	Survival Age to >90 y, <i>n</i> = 362	Comparison Group, <i>n</i> = 355	Survival Age to >90 y, <i>n</i> = 773	Comparison Group, <i>n</i> = 934	Survival Age to >90 y, <i>n</i> = 144	Comparison Group, <i>n</i> = 122
Age at DNA draw, y	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)

Notes: In the CHS, ever smoking was defined as having smoked more than 100 cigarettes or five packs during the participant’s lifetime; hypertension was defined as a systolic blood pressure 140 mmHg or more or a diastolic blood pressure 90 mmHg or more or a history of hypertension and taking antihypertensive medication; diabetes was defined as fasting glucose more than 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/d for a year at any attended examination; total serum cholesterol was measured using an automated enzymatic procedure (44); hypertension was defined as blood pressure 140/90 mmHg or more or on antihypertensive medication; diabetes was defined as fasting blood glucose more than 125 mg/dL, a random blood glucose of more than 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 160 mmHg or more and/or diastolic blood pressure 100 mmHg or more and/or blood pressure-lowering medication with an indication for hypertension; total serum cholesterol was measured using an automated enzymatic procedure (40); diabetes was defined as self-reported diabetes at baseline. In the AGES-Reykjavik, ever smoking was defined as having smoked more than 100 cigarettes in one’s lifetime; total serum cholesterol was measured using an automated enzymatic procedure (40); hypertension was defined as systolic blood pressure 140 mmHg or more, diastolic blood pressure 90 mmHg or more, use of antihypertensive medications, or self-report; diabetes was defined as fasting glucose more than 125 mg/dL, use of insulin or oral hypoglycemic medications, or self-report. AGES-Reykjavik = Age, Gene/Environment Susceptibility-Reykjavik Study; CHS = Cardiovascular Health Study; CVD = cardiovascular disease.

*As a proportion of all deaths for those in the survival to age 90 years or older group.

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 ORs 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; Supplementary Table 3).

Of the 24 strongest independent regions shown in Table 2, the 22 SNPs tested in all four CHARGE cohorts were selected for further evaluation, and 16 were successfully genotyped in the second stage cohorts. Only 1 of the 16 SNPs had a smaller p value after including the replication

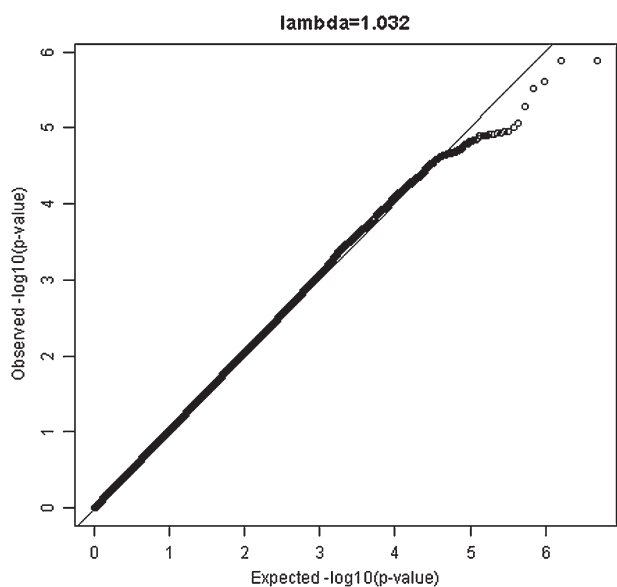


Figure 1. Quantile–quantile plot for the 2,287,520 single-nucleotide polymorphisms in the meta-analysis of survival to age 90 years or older.

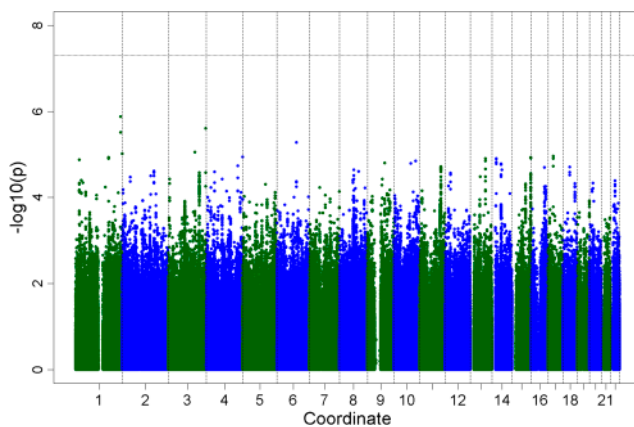


Figure 2. Plot of genome-wide association study for longevity meta-analysis (persons surviving to age ≥ 90 years, $n = 1,836$, and comparison group, $n = 1,955$) showing the $-\log_{10}(p)$ values based on the fixed-effects meta-analysis by chromosome. Line indicates threshold for genome-wide significance of 5×10^{-8} .

Table 2. Top 24 SNP Associations Ranked by *p* Value for Meta-analysis of Four Cohorts for Survival to Age 90 Years or Older (*n* = 1,836) Compared With Survival to Age 55–80 Years (*n* = 1,955)* and Second Discovery Stage Meta-analysis Results

Hit Number	Marker Name	Chromosome	Position	Closest Reference Gene	Distance (bp) From Closest Gene or Function	Alleles			<i>p</i> Value	Study Effect Direction	Number of Supporting SNPs	Effective Sample Size (proportion of total)	Second Discovery Stage Meta-analysis—CHARGE Plus Leiden and Denmark Cohorts		
						Minor	Major	MAF					OR	OR	<i>p</i> Value
1	rs443878	1	238971041	<i>RG57</i>	34,398	T	C	.04	0.41	1.3×10^{-6}	10	2,435	++	0.83	.068
2	rs9825185	3	196415923	<i>C3orf21</i>	Intron	C	A	.13	1.44	2.5×10^{-6}	24	0.64	---	1.10	.045
3	rs954551	6	102886028	<i>GRIK2</i>	262,554	G	A	.25	0.77	5.3×10^{-6}	55	0.88	†	—	—
4	rs7624691	3	138345769	<i>IL20RB</i>	133,159	C	T	.43	0.80	8.8×10^{-6}	67	0.92	++	0.95	.092
5	rs10888267	1	246126046	<i>OR2W3</i>	Missense	C	T	.45	1.25	9.7×10^{-6}	2	0.92	†	—	—
6	rs9972933	17	28589381	<i>ACCN1</i>	Intron	T	C	.23	0.77	1.1×10^{-5}	30	0.91	++	0.89	.003
7	rs2739532	4	190944424			C	G	.27	1.48	1.1×10^{-5}	9	0.99	‡	—	—
8	rs8029244	15	98826098	<i>LASS3</i>	Intron	A	G	.49	0.79	1.2×10^{-5}	108	0.37	++	0.90	.002
9	rs16850255	1	175085164	<i>PAPPA2</i>	6,573	C	T	.21	0.75	1.2×10^{-5}	81	0.86	++	0.92	.041
10	rs1543505	14	22432468	<i>REM2</i>	5,739	G	A	.28	1.27	1.3×10^{-5}	23	0.83	++	1.12	.001
11	rs7321904	13	80681190	<i>SPRY2</i>	868,103	T	C	.07	0.64	1.3×10^{-5}	40	0.93	++	0.92	.179
12	rs17401847	1	20111053	<i>OTUD3</i>	971	G	A	.15	1.38	1.4×10^{-5}	26	0.90	++	1.12	.015
13	rs3124736	10	115487102	<i>CASP7</i>	6,450	A	G	.03	2.30	1.4×10^{-5}	0	0.89	‡	—	—
14	rs690232	9	92422258	<i>DIRAS2</i>	Intron	A	G	.30	1.27	1.6×10^{-5}	68	0.64	†	—	—
15	rs9664222	10	89328613	<i>MINPP1</i>	25,489	A	C	.21	0.77	1.6×10^{-5}	55	0.93	---	0.82	6.77×10^{-7}
16	rs11157721	14	49586464	<i>LOC19691333</i>	655	T	C	.39	0.79	1.7×10^{-5}	53	0.86	++	0.90	.002
17	rs4690810	4	166500130	<i>SCAMOL</i>	16,456	C	T	.35	0.79	1.9×10^{-5}	81	0.85	++	0.93	.044
18	rs11605096	11	113047320	<i>TMPPRSS5</i>	16,162	A	C	.12	0.71	1.9×10^{-5}	94	0.93	†	—	—
19	rs16972414	18	35709920	<i>PIK3C3</i>	2,079,276	G	A	.30	0.79	2.0×10^{-5}	94	0.93	†	—	—
20	rs12935091	16	70082709	<i>ZNF19</i>	1,954	G	A	.07	0.62	2.0×10^{-5}	46	0.94	++	0.80	.002
21	rs210332	14	53262222	<i>BMP4</i>	223,982	C	T	.19	1.33	2.3×10^{-5}	2	0.65	†	—	—
22	rs17369174	8	76128602	<i>CRISPLD1</i>	19,256	G	T	.10	0.69	2.3×10^{-5}	83	0.79	++	0.86	.014
23	rs6721003	2	166931758	<i>SCN7A</i>	38,026	A	G	.45	1.23	2.4×10^{-5}	55	0.91	++	1.09	.006
24	rs4734457	8	101573533	<i>ANKRD46</i>	28,644	A	C	.10	1.75	2.5×10^{-5}	0	0.99	++	1.10	.098

Notes: *p* Values are for the inverse variance-weighted meta-analysis. Distances to genes are given in base pairs. Position is for NCBI Build 36. ORs are for each additional minor allele. Number of supporting SNPs: The number of SNPs within 500 kb of the top SNP that are in LD with the top SNP in the HapMap CEU release 22 ($r^2 \geq .10$) and have association $p < .05$. For imputed and direction, study-specific information is presented in the order: Age, Gene/Environment Susceptibility-Reykjavik Study, Rotterdam Study, FHS, CHS, Leiden, and Denmark. Direction: + = minor allele increases odds of survival more than 90; - = minor allele decreases odds of survival; ? = not tested. CEU = Centre d'Etude du Polymorphisme Humain European Ancestry, CHARGE = Cohorts for Heart and Aging Research in Genomic Epidemiology, CHS = Cardiovascular Health Study; FHS = Framingham Heart Study; LD = linkage disequilibrium; MAF = minor allele frequency; OR = odds ratio; SNPs = single-nucleotide polymorphisms.

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

† Genotyping requested, not completed.

‡ Genotyping not requested.

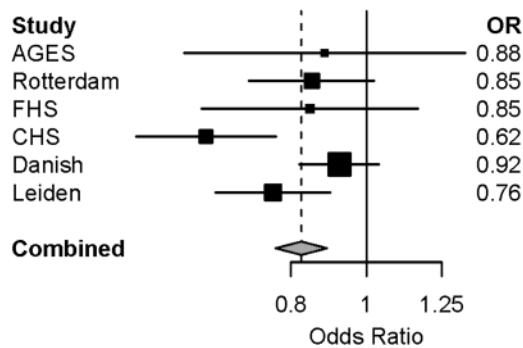


Figure 3. Study-specific odds ratios (ORs) and 95% confidence intervals for *MINPP1* (rs9664222) longevity association.

Note: AGES-Reykjavik = Age, Gene/Environment Susceptibility-Reykjavik Study; CHS = Cardiovascular Health Study; FHS = Framingham Heart Study.

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 ~25 kb 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* = .0014), whereas the Danish study showed a nonsignificant trend in the same direction

(OR = 0.92, *p* = .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 (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 prespecified 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

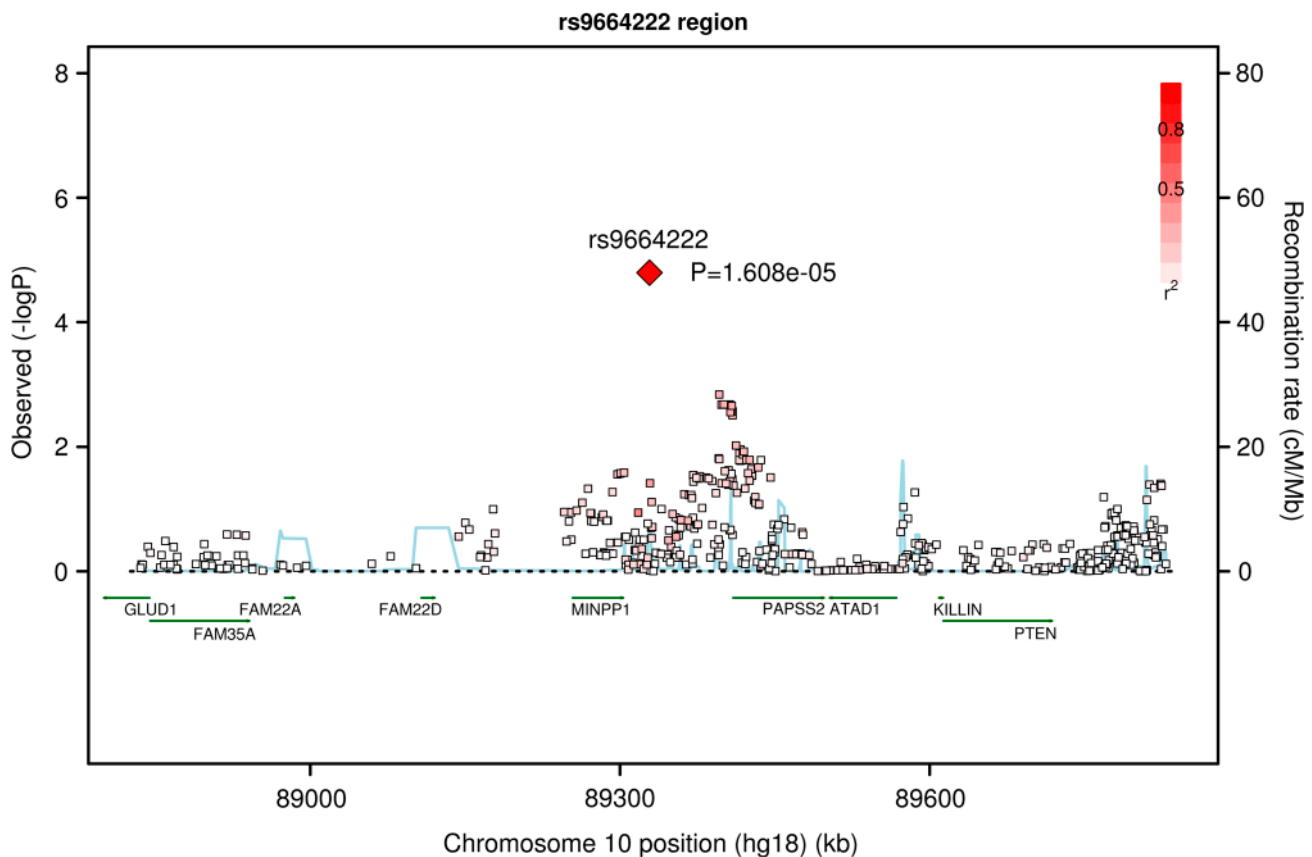


Figure 4. Regional plot for rs9664222 near *MINPP1*.

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 biologic 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 (46). There is no evidence that this SNP is functional; furthermore, its distance from the gene shows that it is not in strong linkage disequilibrium with SNPs in *MINPP1* (47). However, it is well known that important regulatory elements are found outside of genes. This SNP is within 50 kb of two copy number variants. The finding of an 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 (46). The *LASS* gene family contains a group of highly conserved genes that are found in all eukaryotic species. *LASS* isoforms are mammalian homologues of the yeast longevity assurance gene 1, which encodes a protein that regulates life span (48). 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 synthase family, which is important in sphingolipid metabolism, cell differentiation, cell cycling, and apoptosis (46). *LASS3* may be involved in sphingolipid synthesis or its regulation (49).

IL20RB, interleukin 20 receptor beta IL-20, plays a role in skin inflammation and the development of hematopoietic cells (50) and is of interest because of the strong associations of inflammation with the aging process (51). 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 (50). The *ACCN1* gene encodes amiloride-sensitive sodium channels with two hydrophobic transmembrane regions and a large extracellular loop, which has many cysteine residues with conserved spacing (52,53). The member encoded by this gene may play a role in neurotransmission. *ACCN1* was found to be associated with multiple sclerosis (54). Pregnancy-associated plasma protein A2 (*PAPPA2*) is a metalloproteinase regulating local insulin-like growth factor pathway action (55). Genetic deletion extends life span in the mouse by 30%–40% (56) and is characterized by delay in thymic involution (57) and low rates of tumor incidence (56). 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 with 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 descent. 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 versus 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 aiming to identify variants for this phenotype will have to consider small effect sizes and target a sample size larger than our nearly 2,000 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 years. 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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CONFLICT OF INTEREST

The content is solely the responsibility of the authors and does not necessarily represent the views of the NIA, NHLBI, the National Institute of Neurological Disorders and Stroke, or NIH.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at: <http://biomed.gerontologyjournals.org/>

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