

Human longevity is influenced by many genetic variants: evidence from 75,000 UK Biobank participants

Luke C. Pilling¹, Janice L. Atkins¹, Kirsty Bowman¹, Samuel E. Jones², Jessica Tyrrell², Robin N. Beaumont², Katherine S. Ruth², Marcus A. Tuke², Hanieh Yaghootkar², Andrew R. Wood², Rachel M. Freathy², Anna Murray², Michael N. Weedon², Luting Xue³, Kathryn Lunetta^{3,4}, Joanne M. Murabito^{4,5}, Lorna W. Harries⁶, Jean-Marie Robine⁷, Carol Brayne⁸, George A. Kuchel⁹, Luigi Ferrucci¹⁰, Timothy M. Frayling², and David Melzer^{1,9}

¹*Epidemiology and Public Health Group, University of Exeter Medical School, RILD Level 3, Royal Devon and Exeter Hospital, Barrack Road, Exeter, EX2 5DW, UK*

²*Genetics of Complex Traits Group, University of Exeter Medical School, RILD Level 3, Royal Devon and Exeter Hospital, Barrack Road, Exeter, EX2 5DW, UK*

³*School of Public Health, Department of Biostatistics, Boston University, Boston, MA, USA*

⁴*The Framingham Heart Study, Framingham, MA 01702, USA*

⁵*Section of General Internal Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA*

⁶*Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, RILD Level 3, Royal Devon and Exeter Hospital, Barrack Road, Exeter, EX2 5DW, UK*

⁷*Institut National de la Sante et de la Recherche Médicale (INSERM U1198), Montpellier, and Ecole Pratique des Hautes Études (EPHE), Paris, France*

⁸*Cambridge Institute of Public Health, School of Clinical Medicine, University of Cambridge, Cambridge, UK*

⁹*Center on Aging, University of Connecticut, 263 Farmington Avenue, Farmington, CT 06030, USA*

¹⁰*National Institute on Aging, Baltimore, MD 21224, United States.*

Key words: aging, longevity, GWAS, genetic, human

Received: 02/05/16; **Accepted:** 03/10/16; **Published:** 03/23/16

Correspondence to: David Melzer, PhD; **E-mail:** D.Melzer@exeter.ac.uk

Copyright: Pilling et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Abstract: Variation in human lifespan is 20 to 30% heritable in twins but few genetic variants have been identified. We undertook a Genome Wide Association Study (GWAS) using age at death of parents of middle-aged UK Biobank participants of European descent (n=75,244 with father's and/or mother's data, excluding early deaths). Genetic risk scores for 19 phenotypes (n=777 proven variants) were also tested. In GWAS, a nicotine receptor locus (CHRNA3, previously associated with increased smoking and lung cancer) was associated with fathers' survival. Less common variants requiring further confirmation were also identified. Offspring of longer lived parents had more protective alleles for coronary artery disease, systolic blood pressure, body mass index, cholesterol and triglyceride levels, type-1 diabetes, inflammatory bowel disease and Alzheimer's disease. In candidate analyses, variants in the TOMM40/APOE locus were associated with longevity, but FOXO variants were not. Associations between extreme longevity (mother ≥ 98 years, fathers ≥ 95 years, n=1,339) and disease alleles were similar, with an additional association with HDL cholesterol (p=5.7x10⁻³). These results support a multiple protective factors model influencing lifespan and longevity (top 1% survival) in humans, with prominent roles for cardiovascular-related pathways. Several of these genetically influenced risks, including blood pressure and tobacco exposure, are potentially modifiable.

INTRODUCTION

Over the last several decades, genetic studies conducted *in-vitro* and using model organisms have produced a stream of exciting findings linking specific pathways to major effects on aging [1], including, for example, gene knockouts in nutrient sensing pathways that cause dramatic life extension in *C. elegans*. However, humans outlive laboratory models many times over and humans suffer from several conditions that don't normally affect laboratory animals. Moreover, beyond obvious differences in rate of aging, body size and higher brain functions, genomic responses in mice poorly mimic human inflammatory responses [2]. It is therefore uncertain to what extent the laboratory findings and the 'single pathway major effect' model is relevant to human aging and longevity [3]. Additionally, in humans, health behaviors plus social and economic factors play a major role in explaining differences in survival, with smoking being a substantial negative factor [4].

In addition to environmental exposures, genetic variation is important: for example, studies conducted on heterozygotic and homozygotic twins consistently report heritability of longer human lifespans of 20-30% [5,6], with negligible heritability before age 60 years but increasing estimates at advanced ages [7]; the low early heritability is hypothesized to be due to more "accidental" or exposure-related deaths at younger ages. Well-powered genome wide association studies (GWAS) provide robust evidence on which common single nucleotide polymorphisms (SNP) are associated with traits including longevity (selected references: [8–14]).

Deelen et al. recently reported that the T allele of variant rs2149954 (on chromosome 5q33.3) is associated with survival to 90 years of age (combined sample: 12,704 cases vs. 75,374 controls <65 years; combined p -value= 1.7×10^{-8}) [13]. This allele has previously been associated with lower blood pressure. The analysis also confirmed the association between variant rs4420638 (*APOE/TOMM40* locus) and survival to age 90 ($p=3.4 \times 10^{-36}$). In another recent meta-analysis of data from 6,036 participants of European descent who survived beyond 90 years versus 3,757 younger controls (aged 55 to 80 years) [14] no genome wide significant variants were identified. In candidate analyses the *APOE* locus was consistently associated across the participating cohorts, and some evidence was found to support the *FOXO3* gene variants previously linked to longevity in candidate studies in Asian and other populations (best performing variant in discovery analysis rs10457180, uncorrected p -value=0.012 for a

priori SNP rs2802292). In smaller previous studies, the frequency of proven disease risk increasing SNP alleles appeared no different in long lived individuals, which was interpreted as showing that longevity is achieved independent of disease risk alleles [15,16].

Identifying genetic variants associated with longevity using unbiased methods is challenging. As described above, a common 'direct' approach has been to compare older study volunteers with younger participants from the next generation, but this approach may be biased by the many changes in exposures (e.g. early infectious diseases, changing medical treatment) and increasing life expectancy across the generations. Ideal comparisons might be of exceptionally old individuals with those from a representative sample of their own generation who died at younger ages, but obtaining DNA samples from controls who died decades earlier is challenging. An 'indirect' approach to the ideal design, based on the assumption that longevity is partly a genetic transmissible trait, is to access DNA from offspring and test for variations associated with the longevity of their parents. The middle-aged offspring of long-lived parents have less cardiovascular disease, cancer, diabetes, and all-cause mortality compared to offspring whose parents died at younger ages [17], consistent with the inheritance of longevity associated genetic variants. This better health status in offspring showed a linear association with advancing parental age, with a slightly stronger association with mother's compared to father's age at death. Given that offspring inherit their DNA from two parents who might have died at very different ages, associations with longevity in offspring are diluted and samples 3–4 times larger than the direct younger vs older approach are needed. In fact, Tan et al [18] estimate that 1,500 participants of at least one long-lived parent would be needed to achieve >90% power to detect less common alleles (5% frequency) with effects of 0.85 (in binary analysis of offspring of 1 long-lived parent vs. controls) with 95% confidence.

In the current analysis we aimed to identify common genetic variants (prevalence $\geq 1\%$) associated with longer parental lifespan. We focused firstly on a broad range of survival, aiming to identify associations relevant to the interplay of aging and age-related disease (i.e. of relevance to geroscience) [19]. We then also analyzed associations with extreme parental survival or longevity, which we defined as top 1% of survival. To achieve the sample sizes required, we utilized data from UK Biobank. We first performed genome-wide association studies (GWAS) and then used genetic risk scores (GRS) of known variants to test the hypothesis that offspring of longer-lived parents have lower

genetic-risk of common risk traits and diseases. In these analyses we excluded ‘premature’ deaths and included middle aged participants only (age 55 to 70), so that the range of parents’ ages were relevant to ‘normal’ aging. Analyses including younger biobank respondents would include large numbers of parents who were still relatively young for studying longer lifespan, biasing estimates toward early mortality.

RESULTS

Our sample included ‘white’ British UK Biobank participants aged 55-70 years old (n=75,244 with data on fathers survival, mothers survival or both; Table 1). There were relatively few current smokers (8.3% overall), but smoking rates were higher in those with short lived parents. The mean age at death of fathers was 72.9 (range 46 to 105 years) and mothers 78.5 years old (range 57 to 107 years). Nearly half the participants (48.5%) were men. Three continuous phenotypes were utilized throughout this analysis; participant’s father’s age at death (n = 63,775), mother’s age at death

(n=52,776) and combined (normalized) mothers and fathers ages at death (n=45,627 with age at death data for both parents). We had 90.4% power to detect an allele of 5% minor allele frequency in the sample of 45,627 accounting for 0.1% of the variance in the phenotype after multiple-testing correction ($\alpha=5 \times 10^{-8}$).

In addition we created a binary “extreme longevity” phenotype based on the top 1% of the age at death distribution for mothers and fathers (≥ 98 years and ≥ 95 years, respectively). Of 45,627 participants with age at death data for both parents 907 had at least one parent who died within these ranges (1.99%). An additional 432 participants had at least one parent still alive who met the criteria; therefore 1,339 participants had at least one parent (alive or dead) who lived to an “extreme age” (3.2% of 42,273 participants; those with ‘discordant’ parents – one long-lived and one short-lived – are excluded). See Supplementary Figure 1 for a flowchart of the participants included in each of the four primary analyses.

Table 1. Summary statistics for the UK Biobank participants eligible for at least one analysis

Phenotype	All participants			Long-lived group †			Medium-lived group †			Short-lived group †			p-value *
	N ∞	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	
Age at recruitment	75,244	62.077	4.060	8,655	63.529	3.659	21,299	62.862	3.883	25,771	62.096	4.039	3.6E-209
Body mass index (BMI)	75,038	27.654	4.687	8,633	26.970	4.358	21,248	27.537	4.588	25,683	28.040	4.812	2.1E-80
Fathers age at death	63,775	72.925	11.095	7,171	81.844	12.456	21,299	76.973	6.143	23,984	64.396	10.209	n/a
Mothers age at death	52,776	78.473	9.489	6,043	87.215	9.789	21,299	82.130	5.269	21,422	71.808	9.009	n/a
Combined parent age death °	45,627	0.003	1.526	4,052	2.318	0.697	21,299	0.736	0.818	19,635	-1.336	0.978	n/a
Sex (% males)	75,244	48.49%	n/a	8,655	49.10%	n/a	21,299	48.72%	n/a	25,771	48.79%	n/a	8.3E-01
Smoking status (% current)	74,990	8.34%	n/a	8,637	6.24%	n/a	21,237	7.78%	n/a	25,671	8.98%	n/a	7.5E-16
† "Long-lived"=offspring of at least one long-lived parent, "medium-lived"=two medium-lived parents, "short-lived"= at least one short-lived parent. See methods.													
∞ The total N of all participants is greater than the sum of long/medium/short-lived groups as the total includes participants eligible for any analysis (e.g. those with living parents already of extreme age, and participants with age-at-death information on only one parent), whereas the groups only contain participants eligible for analyses including offspring where age-at-death information is available for both parents.													
* p-value from Kruskal-Wallis non-parametric analysis of variance test for significant difference in distribution of phenotype between the 3 longevity categories. No test was performed on parent’s age at death across the categories as the categories are derived from the age at death of the participants’ parents.													
° Z-scored mother's and father's ages at death, summed													

Genome Wide Association Study: smoking-related variants are associated with father's age at death

Of the 9,658,292 genetic variants included none were significantly associated ($p < 5 \times 10^{-8}$) with "parent's age at death, combined" however one locus (36 variants in strong Linkage disequilibrium) on chromosome 15 was associated with "father's age at death," and 1 variant (on chromosome 22) with "mother's age at death" (see Supplementary Table 2 for the top 1,000 results from each GWAS; see Supplementary Figures 2-5 for Manhattan and QQ plots). We focused on the locus on chromosome 15 associated with father's age at death, because the variant associated with mother's age at death was of low frequency (3%) and not in a typical "peak" expected of robust results (see Supplementary Figures 6-7 for LocusZoom plots [20]). rs1051730 is in this loci (beta between G allele and father's age at death = -0.0269, $se = 0.0049$, $p = 3 \times 10^{-8}$); the A allele of rs1051730 in the nicotinic acetylcholine receptor alpha 3 subunit *CHRNA3* gene has been linked to smoking fewer cigarettes and lower risks of lung cancer, although this variant does not influence the chances of starting to smoke [21]. This analysis included fathers who died at ≥ 46 years of age; including only father's aged ≥ 66 (the short-lived cut-off empirically calculated – see methods) reduces the association but it remains significant (beta = -0.0207, $p = 6 \times 10^{-6}$; sample size reduced from 63,775 to 47,094).

We tested rs1051730 against smoking-status (in logistic regressions using binary current/never smoking status as the outcome) in the participants and found that per G allele there is an increased likelihood of current smoking (OR = 1.063, 95% CIs = 1.020 to 1.108, $p = 0.003$). Participants' smoking status is associated with father's age at death (per year OR = 0.993, 95% CIs = 0.991 to 0.996, $p = 2 \times 10^{-6}$), however adjusting for smoking status (current/former/never) in the model of rs1051730 had little effect on the association with father's age at death ($p = 4.7 \times 10^{-8}$).

The association between rs1051730 and mother's age at death (Beta = 0.017, $p = 1.6 \times 10^{-3}$) did not reach genome wide significance in our UK Biobank participants. We tested the association of rs1051730 with father's age at death in the Framingham Heart Study (FHS) generation 2, where 2033 participants were available (mean age at death of the fathers = 77.4 years, SD = 11.5, range = 47.2 to 102.8). The association was non-significant, but was directionally consistent (per C allele coefficient = 0.008, $p = 0.98$). However, the effects size of this SNP is modest and the power to detect the association in the FHS was only 1% (Quanto parameters: coefficient = 0.0269, $n = 2033$, MAF = 0.17, $\alpha = 0.05$).

In the GWAS against the binary "extreme age" phenotype (at least one parent very long-lived) two variants (rs528161076 and rs75824829, on chromosome 7 and 9 respectively; see Supplementary Figures 8-9 for Locus Zoom plots) were found to be significant ($p < 5 \times 10^{-8}$). rs75824829 may be anomalous as it is not in a "peak" of associations expected of robust results (Supplementary Figure 4; Supplementary Figure 9), however rs528161076 is in a distinct peak of variants in an intron of *AP5Z1* (*adaptor related protein complex 5, zeta 1 subunit*, thought to be involved in homologous recombination DNA double-strand break repair). The association between rs528161076 and extreme longevity should still be interpreted with caution, as the variant is not associated with the continuous age at death variables (combined parent's age at death $p = 0.65$, father's $p = 0.47$ and mother's $p = 0.96$).

In all four GWAS performed, no variants on the X or Y chromosomes were associated with the phenotypes ($p > 1 \times 10^{-5}$). For mitochondrial variants, the smallest p-values for mother's age at death, was 9×10^{-3} .

Heritability of parental longevity explained by genotyped common variants

We determined the variance in parental age at death explained by all the common genetic variation (minor allele prevalence $> 1\%$) robustly genotyped directly on the UK Biobank arrays ($n = 457,643$; see methods). For combined parents age at death, the variance attributed to the measured genotypes was 8.47% (SD = 1.06%); for mothers age at death 4.85% (SD = 1.01%) and fathers age at death 5.35% (SD = 1.04%). In a sensitivity analysis including all directly genotyped variants irrespective of minor allele frequency and missingness ($n = 845,997$) the variance attributed for the combined age at death phenotypes was 10.24% (SD = 1.26%); for mothers age at death 6.08% (SD = 1.21%) and fathers age at death 5.79% (SD = 1.23%).

Genetic Risk Score associations

For each genetic risk score (GRS) we first verified the association with the best-fit phenotype corresponding to the reported association: for example the coronary artery disease (CAD) GRS was tested against prevalent coronary heart disease in the offspring (CHD = myocardial infarction or angina) (Table 2). All GRS were associated with their available phenotypes in UK Biobank in the expected direction, for example CAD GRS and CHD (per weighted allele increased OR = 1.12, 95% CIs: 1.10 to 1.13, $p = 1 \times 10^{-64}$). The lipid GRS could not be tested directly, as serum lipid concentrations are not yet available, however all three separate risk scores

are significantly associated with CHD. Although there were only 28 participants with dementia, a significant association was seen with the Alzheimer's disease GRS (per weighted allele OR=1.52, 95% CIs: 1.17 to 1.98, $p=2 \times 10^{-3}$). Telomere length was not assessed in the Biobank participants so validation was not possible.

We next tested associations between each GRS and parental age at death (combined mothers and fathers) in linear regression models plus 'extreme longevity' (at least 1 parent lived to the top 1% of the age at death distribution in UK Biobank) (Figure 1). These analyses included 76 statistical tests (19 GRS against 4 primary longevity phenotypes) and therefore we have 'starred' associations passing Benjamini-Hochberg correction only, although each test has a strong and distinct prior hypothesis (see full results in Supplementary Table 3). We observed associations in the expected directions (i.e. lower disease risk score or higher numbers of relatively protective alleles associated with older ages at death) between the combined parental age at death continuous trait and eight GRS: for CAD (unadjusted $p=1.5 \times 10^{-11}$), LDL cholesterol ($p=1.8 \times 10^{-7}$), CAD without lipid-associated alleles ($p=1.8 \times 10^{-7}$), triglycerides ($p=1.0 \times 10^{-2}$), systolic blood pressure (SBP, $p=1.2 \times 10^{-4}$), BMI ($p=8.2 \times 10^{-4}$), Inflammatory Bowel Disease ($p=7.0 \times 10^{-3}$), T1D (7.5×10^{-3}), and Alzheimer's disease ($p=1.4 \times 10^{-2}$). Crohn's disease ($p=2.3 \times 10^{-2}$) and breast cancer ($p=3.5 \times 10^{-2}$) were associated at nominal significance ($p < 0.05$) but not after multiple-testing correction.

A number of GRS had similar trends for the extreme longevity association but become statistically non-significant in the binary analyses of offspring of long-lived parents, which has reduced statistical power (binary analysis vs. continuous). The HDL cholesterol GRS was associated with extreme longevity ($p=5.7 \times 10^{-3}$) although this association did not reach statistical significance in the linear analysis of continuous age at death.

We observed consistent effect directions, but reduced effect size and significance in separate analyses of mother's age at death compared to father's (Supplementary Table 3); with the exception of Alzheimer's disease (AD) GRS which is only associated with mother's age at death but not father's (coefficient=-0.111, $p=3.9 \times 10^{-4}$; coefficient=-0.001, $p=0.99$, respectively) and Triglyceride level (TG) GRS was only associated with fathers not mothers age at death (father's coefficient=-1.366, $p=9 \times 10^{-4}$; mother's coefficient=-0.183, $p=0.63$, respectively). In linear regression models for AD and TG GRS there were no statistical interactions between mothers and fathers ages at death ($p > 0.05$).

To illustrate combined effect sizes, we computed an estimate across the three GRS most robustly associated with parental longevity (unadjusted $p < 0.001$): CAD, SBP and LDL. In total, 525 participants (of 45,627) were in the bottom quintile (20%) for all three GRS;

Table 2. Genetic Risk Score associations with corresponding phenotypes in 75,244 participants included for at least one analysis

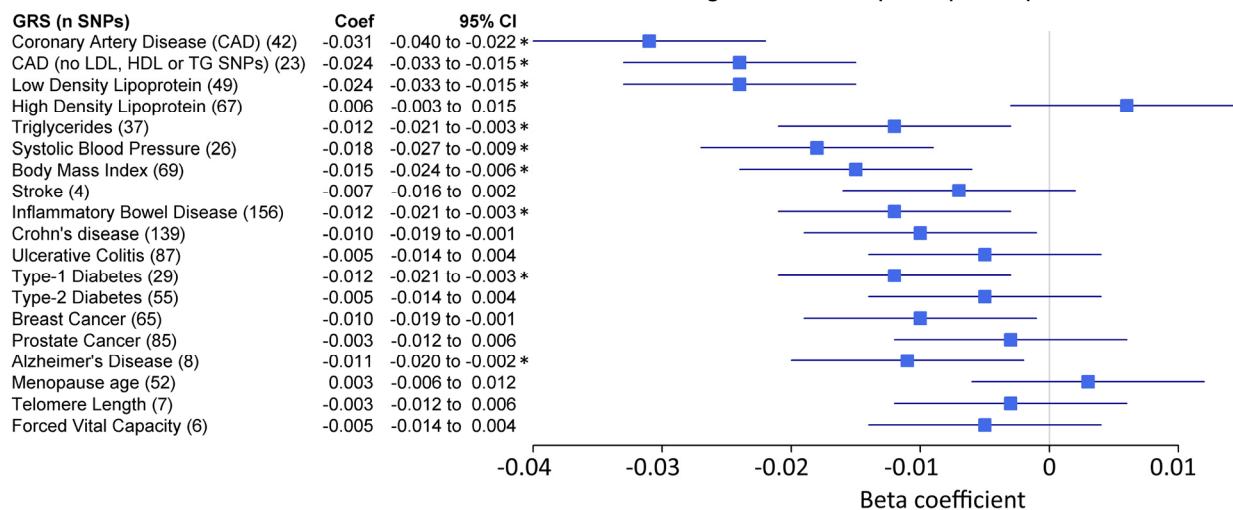
Model	GRS (n SNPs)	Phenotype (n cases)	OR (95% CIs)	p-value
<i>Logistic regression models</i>				
	Coronary Artery Disease, CAD (42)	Prevalent CHD (5067)	1.12 (1.10 to 1.14)	6.4×10^{-55}
	CAD, without LDL, HDL and TG SNPs (23)	Prevalent CHD (5067)	1.13 (1.11 to 1.16)	2.3×10^{-36}
	LDL cholesterol (49)	Prevalent CHD (5067)	2.97 (2.40 to 3.68)	1.3×10^{-23}
	HDL cholesterol (67)	Prevalent CHD (5067)	0.62 (0.50 to 0.78)	3.1×10^{-05}
	Triglycerides, TG (37)	Prevalent CHD (5067)	1.88 (1.43 to 2.46)	5.3×10^{-06}
	Stroke (4)	Prevalent stroke or TIA (1790)	2.04 (1.03 to 4.06)	4.0×10^{-02}
	Type-1 Diabetes (29)	Type-1 diabetes diagnosis (55)	2.35 (1.98 to 2.78)	9.8×10^{-23}
	Type-2 Diabetes (55)	Type-2 diabetes diagnosis (4,052)	5.29 (4.55 to 6.16)	1.7×10^{-103}
	Alzheimer's Disease (8)	Dementia diagnosis (28)	1.52 (1.17 to 1.98)	1.9×10^{-03}
	Inflammatory Bowel Disease (156)	Inflammatory Bowel Disease (654)	1.13 (1.10 to 1.16)	5.3×10^{-22}
	Crohn's Disease (139)	Prevalent Crohn's disease (250)	1.16 (1.11 to 1.21)	1.1×10^{-12}
	Ulcerative Colitis (87)	Prevalent ulcerative colitis (414)	1.17 (1.13 to 1.22)	4.4×10^{-15}
	Prostate Cancer (85) *	Prevalent prostate cancer (863)	1.18 (1.16 to 1.21)	2.5×10^{-52}
	Breast Cancer (65) *	Prevalent breast cancer (2036)	1.12 (1.11 to 1.14)	2.3×10^{-43}
	Telomere Length (7)	n/a		
<i>Linear regression models</i>			Coefficient (95% CIs)	p-value
	BMI (69)	Body mass index	7.07 (6.62 to 7.51)	2.8×10^{-209}
	Systolic Blood Pressure (26)	Systolic blood pressure	1.31 (1.14 to 1.47)	4.3×10^{-57}
	Age at menopause (52) *	Age at menopause	-1.79 (-1.92 to -1.65)	2.4×10^{-154}
	Forced Vital Capacity (6)	Forced Vital Capacity	0.001 (0.001 to 0.002)	7.0×10^{-09}

GRS = genetic risk score. OR = per weighted allele odds ratio. CIs = confidence intervals.

these participants are the “lowest risk” group for these three cardiovascular traits. Correspondingly, 524 participants were categorized as having the “highest risk” (top 20% of genetic risk for all three traits). In adjusted logistic regression models, participants with the lowest genetic risk (highest number of protective alleles) had 3.25 times the likelihood of having at least

1 parent in the top 1% of ages at death, compared to those with the highest genetic risk (OR=3.25, 95%CI=1.3 to 8.1, p=0.012). Unadjusted prevalence: 19 participants (1.4% of 1,339) with at least one long-lived parent were in the bottom 20% for all three risk scores, where only 7 participants (0.5% of 1,339) were in the top 20% for all three risk scores.

A. The associations between each GRS and combined age-at-death of participant's parents



B. The associations between each GRS and the top 1% of age-at-death of participants' parents

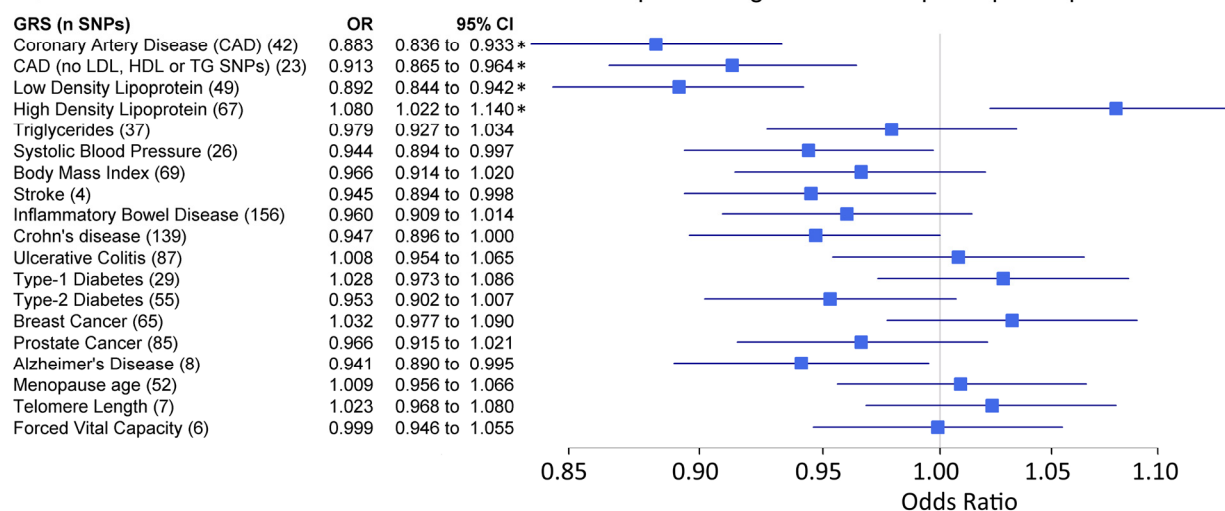


Figure 1. Genetic risk score associations with parents age at death. Forest plots show the relationship between the genetic risk scores (GRS, z-transformed) and 2 different parental longevity traits. Results from regression models (see methods). * indicates the association is significant after adjustment for multiple testing. **(A)** Linear regression results for combined parent's age at death. N=45,627. Coefficients ('coef') show the standard deviation (SD) difference in GRS per SD of parent's age at death. The results for 'CAD (no Lipids)' and 'LDL cholesterol' only appear identical due to rounding; the effects were very similar. **(B)** Logistic regression results for the binary trait "participants of at least one parent reaching the top 1% of the age-at-death distribution vs. participants who parents did not". Discordant participants (i.e. one long-lived and one short-lived) were excluded. n=42,273. n=1,339 participants of at least one long-lived parent (mother aged ≥98 years or father aged ≥95 years). Includes participants with alive parents reaching these limits. Odds Ratios (OR) show the likelihood of participants having at least 1 parent reaching the top 1% of age-at-death distribution per SD difference in GRS.

In a sensitivity analyses we removed variants mapped to the *APOE* locus from the risk scores (rs4420638 was included in the scores for CAD, LDL, HDL and AD) and assessed the associations with the parental age-at-death phenotypes. After exclusion the AD risk score is no longer associated with any of the parental age at death phenotypes. The effect sizes for CAD, LDL and HDL are attenuated but remain significant (see Supplementary Figure 10). Adjusting for presence of at least one ApoE-ε4 allele in the models against continuous age-at-death has the same effect on the associations: the CAD GRS association changes from (coef= -0.023, p= 1×10^{-10}) to (coef= -0.022, p= 2×10^{-9}); the LDL GRS association changes from (coef= -0.28, p= 6×10^{-8}) to (coef= -0.22, p= 1×10^{-4}); the HDL GRS association changes from (coef= 0.078, p=0.15) to (coef= 0.050, p=0.37); the AD GRS association changes from (coef= -0.015, p=0.008) to (coef= 0.002, p= 0.79).

Candidate genetic variants associated with longevity

We have highlighted the GWAS results for a number of SNPs *a priori* selected from the literature (see Supplementary Table 4 for the full details). We found significant (p<0.05, not ‘genome-wide’ significant p< 5×10^{-8}) associations between 4 of the 5 *APOE/TOMM40* SNPs tested and parents age at death (rs2075650 p= 1×10^{-4} , rs429358 p= 8×10^{-7} , rs7412 p=0.02, rs4420638 p= 3×10^{-5} , but not rs405509 p=0.06). These variants are in moderate linkage disequilibrium (R^2 from 0.1 to 0.7). Of 12 *FOXO3* variants assessed, none were significantly associated with a longevity phenotype (p>0.05). The 5q33 variant identified by Deelen et al. [13] is associated with combined parents age at death and father’s, but not mother’s age at death or extreme survival (p=0.005, p=0.01, p=0.1 and p=0.6, respectively). We observed modest associations with 2 of 7 variants at the *CDKN2A* locus (variants previously associated with coronary heart disease) with parent’s age at death (rs1333049 G allele beta=0.015, p=0.0015; rs4977574 A allele beta=0.014, p=0.0018); the *CDKN2A* variants associated with type-2 diabetes were not associated with parent’s longevity. Four variants were reported by Fortney to be associated with exceptional longevity; one is the *APOE* variant already discussed, and the other three are rs4977756 (*CDKN2B/ANRIL*), rs3184504 (*SH2B3/ATXN2*) and rs514659 (*ABO*). Only rs3184504 is associated with extreme longevity in this analysis (T allele beta=-0.0019, p=0.024), but rs4977756 was associated with continuous parent’s age at death (G allele beta=0.017, p=0.025). The final SNP is not associated with any longevity phenotypes in our analysis.

Presence of at least one ApoE-ε4 allele (10,237 of 44,574 participants included in the analysis against continuous parents age at death, after excluding 1,053 ε2/ε4 participants) was associated with reduced age at parents death (coef= -0.088, 95% CI= -0.12 to -0.05, p= 3×10^{-7}) and reduced likelihood of having a parent in the top 1% of the age-at-death distribution (OR=0.78, 95% CI=0.66 to 0.91, p=0.002), compared to participants without an ε4 allele. Presence of at least one ApoE-ε2 allele (5,700 of 44,574) was associated with continuous parents age-at-death (coef= 0.052, 95% CI=0.01 to 0.09, p= 0.014) but not with having a parent in the top 1% of the age-at-death distribution (OR=1.09, 95% CI=0.91 to 1.31, p=0.37).

DISCUSSION

We report the largest analysis thus far of common genetic variants and normal aging related human longevity, based on the indirect approach of testing variant associations in offspring. We have excluded premature deaths and the large number of younger biobank respondents whose parents were likely to be too young and who might bias results toward early deaths. We identified a genome-wide significant variant in the smoking-related *CHRNA3* gene with father’s longevity. We also identified a variant associated with extreme longevity, in the *AP5Z1* gene locus, a gene encoding a homologous recombination DNA double-strand break repair helicase protein [22], although this association with longevity appears less robust. In addition, we have shown that longevity is associated with having greater numbers of relatively protective alleles (i.e. lower genetic risk scores) for several cardiovascular traits including lipid levels, CAD (excluding variants associated with LDL and HDL), BMI, and blood pressure. Weaker associations were also found for risks scores for Alzheimer’s disease and autoimmune diseases. While laboratory model work has shown major longevity effects of single genes or single pathways, our results tend to support a model of human longevity influenced by multiple smaller effect protective and risk variants in several pathways, most prominently in cardiovascular related phenotypes. Our findings also supports the Geroscience concept that chronic diseases and biological processes involved in aging and longevity share similar mechanisms and pathways; efforts to target them may help both delay or prevent the onset of chronic diseases while also increasing longevity [23].

The *CHRNA3* rs1051730 variant has previously been linked to smoking fewer cigarettes and lower risks of lung cancer [21], although this variant does not influence the chances of starting smoking. Our finding

of association with father's age at death is highly plausible given the strongly adverse effect of smoking on survival. The fact that this SNP is also associated with mother's age at death may support this finding, with the weaker (non-genome wide significant) association perhaps explained by the lower rates of smoking in women in the generation of parents being studied. Data on parental smoking and cause of death are not present in the Biobank data so we cannot assess this, however rs1051730 is associated with smoking in the participants ($p=0.003$, current vs. never) and participants smoking is associated with father's age at death ($p=2 \times 10^{-6}$). Adjusting for participants' smoking status in the model of rs1051730 against father's age at death did not change the association. We have noted that our attempt to test this variant independently in the Framingham Heart Study was not successful, but this analysis is severely underpowered, underlining the relatively small effect of this SNP on father's longevity. Additionally, rs1051730 is located in a cluster of variants in high linkage disequilibrium covering several genes so the causal association could feasibly be via another mechanism (see Supplementary Figure 6).

We also analyzed associations with extreme longevity (here defined as at least one parent in the top 1% of age at death, ≥ 98 years for mothers or ≥ 95 years for fathers). The GWAS analysis revealed two suggestive genetic variants that require further study; rs528161076 is in a distinct peak of variants in an intron of *AP5Z1*, a gene encoding a homologous recombination DNA double-strand break repair helicase protein [22], and rs75824829 on chromosome 9 is ~50k from the nearest gene *C9orf62* (*chromosome 9 open reading frame 62*) for which little information is available. For the *AP5Z1* variant, this involves a C insertion after the T allele, with a prevalence 4.5% with at least one copy and is enriched amongst the those with top 1% longevity parents: of 1,335 participants with at least one long-lived parent, 84 have at least one of these insertions (6.3%) compared to 4.39% (1,796 of 40,871) of controls. Finding big enough independent samples to confirm these associations will be challenging.

For the genetic risk score analysis, several associations with continuous longevity become non-significant for extreme longevity, apparently due to lower sample sizes, but the pattern of GRS associations for both phenotypes were similar (Figure 1). More extreme definitions of longevity might yield different results but numbers of centenarian parents, for example, were too small to study. A possible exception to the generally similar risk pattern is the HDL cholesterol GRS, which was associated with extreme longevity but did not reach significance with continuous longevity. A number of

other traits including type-1 diabetes GRS were close to significance, but these require additional study in a larger sample.

Associations between germline genetic variants and phenotypes provide strong evidence of causality because variants are inherited at the beginning of life, before any confounding by environmental or other factors can occur. Our results confirm the importance of several potentially manageable risk factors in achieving exceptional longevity, including adverse lipid levels, raised blood pressure (systolic and diastolic), adiposity (body mass index) and also smoking, as noted above. Although there has been some debate on whether there are paradoxical associations between obesity risks factors and survival in old age [24], our result suggests that genetically influenced life-long exposures in the expected directions (i.e. non-paradoxical effects) do influence human survival. This genetic evidence supports recent work on BMI and survival in a sample of 1 million older people, suggesting that paradoxical associations reflect reverse causation from weight loss in older people who already have major diseases associated with weight loss (Bowman et al, *in review* 2016).

Comparison with previous work

There is consistent epidemiological evidence for a relationship between longevity and lower rates of type-2 diabetes [17], yet previous evidence from the Leiden study ($n=2415$) and the Life Long Family Study ($n=1562$ in generation 1; $n=3102$ generation 2) suggested that offspring of long-lived participants do not have lower burden of type-2 diabetes risk alleles compared to their partners [16,25]. We also did not observe an association between the type 2 diabetes GRS and longevity in the UK Biobank participants, although small effects cannot be excluded.

There is evidence to suggest that long-lived individuals have reduced prevalence of Alzheimer's increasing alleles [16], which includes the *TOMM40/APOE* locus identified in multiple analyses of longevity [13,14,12,10]. We confirmed that the A allele of rs2075650 was positively associated with increased parental age at death ($\beta=0.039$, $p=1 \times 10^{-4}$), however there was no significant association with "extreme longevity" (at least one parent in the top 1% of ages at death). The A allele, here associated with increased parents lifespan, is associated with decreased risk of dementia [26]. Presence of the ApoE- $\epsilon 4$ was associated with reduced likelihood of having a parent in the top 1% of survival ($OR=0.78$, $p=0.002$), consistent with previous reports on mortality risk [27]; however we do

not see a significant increase with presence of an $\epsilon 2$ allele (OR=1.09, $p=0.37$). We find that a variant on chromosome 5q33.3 previously associated with survival to age 90 [13] is associated ($p=0.003$) with combined parental age at death in a consistent direction (T allele associated with increased age at death), but not with extreme survival ($p=0.6$) in this analysis.

A recent study found that longevity-associated loci are enriched for disease-associated loci, consistent with our findings in Biobank [28]. The authors also performed a disease-weighted GWAS analysis, reporting 4 loci associated with exceptional longevity (≥ 90 years) with replication. We replicate the association between rs3184504 (mapped to *SH2B3/ATXN2*), previously shown to be associated with celiac disease [29], in our extreme longevity phenotype, but not the other three variants (although they are associated with continuous parent's age at death $p<0.05$). rs3184504 has also been associated with blood pressure and cardiovascular disease [30]. This suggests rs3184504 is associated with survival to exceptional ages, but the others require further evidence.

Previous analyses have found that long-lived individuals have lower low-density lipoprotein genetic risk than young controls [31,32], which we also observe (offspring of long-lived parents have lower LDL-increasing genetic risk score). Our results extend this work by showing associations also with triglyceride and HDL genetic risk scores, plus BMI and systolic blood pressure, as well as associations with non-lipid cardiovascular disease genetic risk traits.

Limitations

This study is limited to white British UK Biobank participants of Caucasian genetic descent, thus the results may not be applicable to other populations. We plan to address this in future collaborations. Evidence from GWAS studies identifying novel markers is strongest when associations are shown to replicate in independent samples, but unfortunately no large-scale replication resources are currently available. We have therefore been very cautious in reporting the novel markers, which await validation. However, the results for previously proven SNPs (i.e. those with a high prior probability) are likely to be very robust, given our large sample size.

UK Biobank is a volunteer study that did not aim for population representativeness at baseline, although efforts were made to recruit a heterogeneous sample by varying geographic placement of examination sites, including in economically deprived areas; the final response rate was 5.47% [33]. It has been reported that

with sufficient variation in the phenotypes being studied, results are generalizable to the wider UK population [34].

We are limited by the coverage of the genotyping microarray utilized; $\sim 800,000$ genetic variants were directly measured, allowing imputation of 73 million (9 million of which were common enough and high quality enough for inclusion in this GWAS). Many variants may exist outside of the data available for this study, in particular on the X, Y and mitochondrial chromosomes, for which imputed data are not available.

We have studied the normal range of parental ages at death, as well as extreme longevity (top 1% of survival). Some would argue that normal age at death is necessarily influenced by disease and has little relevance to aging. However, age is the major risk factor for most causes of death in later life, and the geroscience view sees aging as a major contributing factor to these diseases and deaths. Our extreme survival group is older than those studied in many of the previous GWAS of longevity [13,14], but did suffer from a somewhat limited sample size of $n=1339$: analyses with bigger samples of those attaining extreme ages are needed. Our longevity phenotypes do involve some repeat testing of the same individuals, but these phenotypes are closely related rather than being independent, and therefore should not have produced additional multiple testing problems.

We excluded early parental deaths based on an empirical approach [17,35] to avoid using arbitrary cutoffs. Only 13.6% of the fathers were aged between 46 and 60 at death (only 2% 46 to 50), suggesting that the exact cut-offs for premature deaths would have a modest effect on findings, especially as the results were similar for the extreme longevity group comparisons.

The available information about the parents is very limited, with no dates of birth for those who had died and no cause of death information. We have excluded UK Biobank participants aged less than 55 to avoid adding large numbers of parents who are likely to be too young to have reached the longer lifespans which are our main focus in this analysis. The lack of cause of death data may have resulted in an underestimation of effect sizes on lifespan due to aging and related disease, as we have had to include e.g. accidental deaths unrelated to aging. It is unlikely that e.g. Second World War related parental deaths could have biased results, as few combatants were aged over 45, our earliest age of death for inclusion the analyses. The consistency of known allele results between normal and extreme ages at death also suggests that our findings are not driven by early deaths.

When comparing the effect sizes between different GRS, it is important to consider that each GRS explains a different proportion of its associated phenotype: this will in turn affect the interpretation of the association with longevity. For instance, only approximately 1% of the variance in mean length of the telomeres is explained by the 7 reported SNPs [36], whereas more than 10% of the variance in the blood lipids is accounted for by associated variants [37]; therefore the strength of association between these two phenotypes and longevity may not be proportionately represented by the effect sizes of the GRS longevity associations.

Conclusions

Longer lifespans in our studied Caucasian origin sample are influenced by multiple common risk and protective genetic variants. Cardiovascular trait variants are particularly prominent in associations with longevity. Several of these genetically influenced risks including tobacco exposure are potentially modifiable. Further work is needed in other ethnic groups and to test less common variants for associations with longevity.

METHODS

Between 2006 and 2010, 503,325 volunteers (aged 45 to 69 years old) were recruited from across the United Kingdom to the UK Biobank study [38]. Of these, 75,244 participants met the inclusion criteria for at least one analysis: participants aged between 55-70 years with complete genetics data and date of death data for either parent (additional participants were included if they had an alive parent that met the “long-lived” criteria for binary analyses). We chose this 55 to 70 age-range because usual intergenerational age gaps mean that very few participants below age 55 at baseline could have parents old enough to be longer lived, thus including younger ages would enrich and potentially bias our study toward premature and early deaths. Participants were excluded if they reported themselves as adopted, or if either parent died prematurely (fathers <46 years or mothers <57 years – see methods below), or if their parent was still alive but not yet long-lived. Several longevity phenotypes are defined below based on the age at death of the participant’s parents.

Parental age at death and longevity phenotypes. Participants were asked the age at which their parents had died (or their current age if still alive). Analyses were performed separately on mother’s age at death and father’s age at death, and also on a combined phenotype. To reduce the effect of higher ages at death

of mothers (compared to the fathers) we first z-transformed the mothers and fathers age at deaths before combining the z-scores into a single summed phenotype. Offspring of parents who died prematurely were excluded because the cause of death of the participant’s parents was not asked, so we could not exclude accidental deaths explicitly. To determine the premature age at death cut-offs we used previously described methods to define the normal range of age at death for mothers and fathers separately, and excluded participants below these values (methods described here [17], description of method applied to UK Biobank here (Atkins et al. in review 2016)). In brief, non-linear least square regression models are used to fit a normal curve to the right-side of the distribution of parents age at death; the left half of the curve is then fitted and cut-offs determined (see [35] and [17])._The analysis identified the following cut-points for short, intermediate, and long-lived parents ages at death: for mothers (57 to 72 years, 73 to 92 years, and ≥ 93 years, respectively) and for fathers (46 to 65 years, 66 to 89 years, and ≥ 90 years, respectively). Parents below the “short” definition are excluded as premature deaths.

We also defined a binary trait for an “extreme longevity” phenotype by determining the top 1% of age at death for mothers (≥ 98 years) and fathers (≥ 95 years) separately. We were able to increase the number of “long lived” parents in all the binary phenotypes by including participants who had responded to the question regarding the age of their parent if still alive. Participants with one long-lived and one short-lived parent were excluded from this analysis.

Finally, we defined two further binary traits for sensitivity analyses testing the consistency of results using a non-linear definition: offspring of at least one long-lived parent vs. offspring of two intermediate-lived parents, and offspring of at least one long-lived parent vs. offspring of at least one short-lived parent (very low numbers of participants with either “both long” or “both short” meant we used the aforementioned “at least one...” definitions). Supplementary Figure 1 contains a flow-chart showing how the phenotypes are derived and the numbers included in each analysis.

UK Biobank genetics data. We used genetic data available (autumn 2015) from 120,286 participants identified as ‘white British’ through self-report and verified through principal components analysis based on genotypes. Kinship coefficients were estimated and related individuals (3rd degree or higher) were removed to provide the maximal unrelated set of individuals. The central UK Biobank analysis team performed these analyses. Details of principal component analyses and kinship analyses can be found in the official UK

Biobank genotyping document (http://biobank.ox.ac.uk/crystal/docs/genotyping_gc.pdf; accessed 1st December 2015).

We used imputed genotypes available from the UK Biobank for association analyses. Briefly, phasing of individuals was carried out using SHAPEIT-2. Imputation was performed using IMPUTE2 and a combined 1000 Genomes / UK10K reference panel. Full details can be found in the official UK Biobank imputation document (http://biobank.ox.ac.uk/crystal/docs/impute_ukb_v1.pdf; accessed 1st December 2015). After filtering for variants with MAF $\geq 1\%$, missingness $< 1.5\%$, imputation quality > 0.4 and with Hardy-Weinberg equilibrium (HWE) $P > 1 \times 10^{-6}$ within the white British participants, 9,658,292 imputed autosomal variants were eligible for the analyses.

We also utilized data directly from the microarrays for variants on the X ($n=19,381$) and Y ($n=284$) chromosomes, and on the mitochondrial genome ($n=135$), which were unavailable in the imputed dataset.

Within “white British” principal components. We selected 95,535 independent SNPs (pairwise $r^2 < 0.1$) directly genotyped with a minor allele frequency (MAF) $\geq 2.5\%$ and missingness $< 1.5\%$ across all UK Biobank participants with genetic data available at the time of this study ($n=152,732$), and with HWE $P > 1 \times 10^{-6}$ within the white British participants. Principal components were subsequently generated using FlashPCA and the first five adjusted for in all analyses [39].

Power calculations. Quanto software version 1.2.4 was utilized for power calculations with parameters: continuous, additive model, mean/SD of standardized outcome=0/1 [40].

Genome Wide Association Study. We used BOLT-LMM to model the associations between imputed variants (dosages) and each phenotype [41], which uses a linear mixed effects model approach. We looked at the results for variants with imputation quality > 0.4 , HWE p -values $> 1 \times 10^{-6}$ and minor allele frequencies $> 0.1\%$ in the white/British subset used for all analyses. For variants on the X, Y and mitochondrial chromosomes only in the directly-genotyped data we used Plink (v1.9) [42] in linear (additive) or binary (fisher) models, as appropriate, adjusted for the same covariates as above including the first 5 principal components from FlashPCA.

Independent testing of GWAS results. We obtained estimates of our genome wide significant results for fathers age at death GWAS in the Framingham Heart

Study (FHS) generation 2 [43], with data on $n=2033$ offspring available. The inclusion criteria and model specification were the same as described for Biobank, with the exception that family structure was taken into account (using R package ‘pedigree’).

Genetic heritability estimation. To estimate the variance in mean parental age at death (and age at death of mothers/fathers considered separately) explained by common genetic variants we utilized the BOLT-REML package [44]. This package uses Restricted Maximum Likelihood (REML) methods for variance component estimation, in this case on the genetics data. We used SNPs that met the following criteria in the 120,286 individuals; variants were excluded if not in Hardy-Weinberg Equilibrium (HWE) ($P < 1 \times 10^{-6}$), or had a minor allele frequency $< 1\%$, or an overall missing rate $> 1.5\%$ in any individual batch, or were on the Y chromosome. This resulted in 457,643 directly genotyped variants for inclusion. We also performed an additional sensitivity analysis relaxing the exclusion criteria to include all 845,997 variants. BOLT-REML determines the “heritability” of phenotypes based on the variance components of the genetics data provided. Prior to analyses, phenotypes are adjusted (by taking the residuals from a linear regression analysis) for confounding factors (age, sex, array type, assessment center, principal components 1-5).

Genetic Risk Score (GRS) creation. For each trait we identified the most recent GWAS meta-analysis and downloaded the results tables. We selected the SNPs identified at genome wide significance ($p < 5 \times 10^{-8}$) and extracted the corresponding genotype information from the imputed data in the UK Biobank data. For all SNPs included in a GRS we checked for high imputation quality (> 0.9), no significant deviation from HWE ($p > 1 \times 10^{-6}$) and low missingness ($< 5\%$ sample missing). We used the imputed genotype (dosage) information and effect size (from the meta-analysis) with Plink (v1.9) function ‘scores’ to generate the weighted GRS for each trait in each participant [42]. The Plink function uses genotype coding for each participant as 0, 1 or 2 trait-increasing alleles, which is multiplied by the effect size (coefficient or odds ratio) from the published study. The resulting “weighted allele score” is summed for all variants associated with a particular trait. See the accompanying Supplementary Methods document for the definition of each genetic risk score, and Supplementary Table 1 for the variants and effect sizes used to create the scores.

Statistical analysis of Genetic Risk Scores. Generalized linear regression models were used throughout (R v3.2.0), with adjustment for age, sex, array type

(‘axiom’ or ‘bileve’), assessment center (22 possible categories), and the first 5 genetic principal components. The regression linker functions ‘logit’ or ‘Gaussian’ were used respectively for logistic and linear outcomes. Forest plots comparing the associations between different GRS’ and each outcome were generated using the R package ‘rmeta’ (v2.16). For these analyses the GRS was first z-transformed so that the statistics reported could be compared between each GRS on the forest plot. In the rest of the manuscript, we refer to the unstandardized, “per weighted allele” effects. In all respects except for the z-transformation the models were identical. Benjamini-Hochberg p-values are calculated to correct for potential false-positive associations (19 GRS against 4 primary longevity phenotypes).

ApoE haplotype definition. The ApoE haplotype is defined using two genetic variants (rs429358 and rs7412). We created a binary phenotype “any $\epsilon 4$ alleles vs. rest” where $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ participants were grouped together ($\epsilon 2/\epsilon 4$ participants were excluded), and the $\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$ and $\epsilon 3/\epsilon 3$ alleles were the control participants. A second phenotype “any $\epsilon 2$ alleles vs. rest” is defined correspondingly.

Funding

This work was generously funded by an award to DM, TF, AM, LH and CB by the Medical Research Council MR/M023095/1. This research has been conducted using the UK Biobank Resource, under application 1417. The authors wish to thank the UK Biobank participants and coordinators for this unique dataset. S.E.J. is funded by the Medical Research Council (grant: MR/M005070/1). J.T. is funded by a Diabetes Research and Wellness Foundation Fellowship. R.B. is funded by the Wellcome Trust and Royal Society grant: 104150/Z/14/Z. M.A.T., M.N.W. and A.M. are supported by the Wellcome Trust Institutional Strategic Support Award (WT097835MF). R.M.F. is a Sir Henry Dale Fellow (Wellcome Trust and Royal Society grant: 104150/Z/14/Z). A.R.W. H.Y., and T.M.F. are supported by the European Research Council grant: 323195:GLUCOSEGENES-FP7-IDEAS-ERC. The funders had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The Framingham Heart Study is supported by Contract No. N01-HC-25195 and HHSN268201500001I and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). The phenotype-genotype association analyses were supported by National Institute of Aging R01AG29451.

This work has made use of the resources provided by the University of Exeter Science Strategy and resulting Systems Biology initiative. Primarily these include high-performance computing facilities managed by Konrad Paszkiewicz of the College of Environmental and Life Sciences and Pete Leggett of the University of Exeter Academics services unit.

Conflict of interest statement

All authors declare no conflicts of interest.

REFERENCES

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013; 153:1194–1217.
2. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker H V, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A*. 2013; 110:3507–3512.
3. Melzer D, Frayling TM, Murray A, Hurst AJ, Harries LW, Song H, Khaw K, Luben R, Surtees PG, Bandinelli SS, Corsi A-M, Ferrucci L, Guralnik JM, et al. A common variant of the p16(INK4a) genetic region is associated with physical function in older people. *Mech Ageing Dev*. 2007; 128:370–377.
4. Ostbye T, Taylor DH, Krause KM, Van Scoyoc L, Taylor Jr. DH, Krause KM, Van Scoyoc L. The Role of Smoking and Other Modifiable Lifestyle Risk Factors in Maintaining and Restoring Lower Body Mobility in Middle-Aged and Older Americans: Results from the HRS and AHEAD. *J Am Geriatr Soc*. 2002; 50:691.
5. Herskind AM, McGue M, Holm N V, Sørensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900. *Hum Genet*. 1996; 97:319–323.
6. Mitchell BD, Hsueh WC, King TM, Pollin TI, Sorkin J, Agarwala R, Schäffer AA, Shuldiner AR. Heritability of life span in the Old Order Amish. *Am J Med Genet*. 2001; 102:346–352.
7. Hjelmborg JB, Iachine I, Skytthe A, Vaupel JW, McGue M, Koskenvuo M, Kaprio J, Pedersen NL, Christensen K. Genetic influence on human lifespan and longevity. *Hum Genet*. 2006; 119:312–321.
8. Sebastiani P, Solovieff N, Dewan AT, Walsh KM, Puca A, Hartley SW, Melista E, Andersen S, Dworkis D a, Wilk JB, Myers RH, Steinberg MH, Montano M, et al. Genetic signatures of exceptional longevity in humans. *PLoS One*. 2012; 7:e29848.
9. Yashin AI, Wu D, Arbeev KG, Ukraintseva S V. Polygenic effects of common single-nucleotide polymorphisms on life span: when association meets causality. *Rejuvenation Res*. 2012; 15:381–394.
10. Nebel A, Kleindorp R, Caliebe A, Nothnagel M, Blanché H, Junge O, Wittig M, Ellinghaus D, Flachsbarf F, Wichmann H-E, Meitinger T, Nikolaus S, Franke A, et al. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. *Mech Ageing Dev*. n.d.; 132:324–330.
11. Malovini A, Illario M, Iaccarino G, Villa F, Ferrario A, Roncarati R, Anselmi CV, Novelli V, Cipolletta E, Leggiero E, Orro

- A, Rusciano MR, Milanesi L, et al. Association study on long-living individuals from Southern Italy identifies rs10491334 in the CAMKIV gene that regulates survival proteins. *Rejuvenation Res.* 2011; 14:283–291.
12. Deelen J, Beekman M, Uh H-W, Helmer Q, Kuningas M, Christiansen L, Kremer D, van der Breggen R, Suchiman HED, Lakenberg N, van den Akker EB, Passtoors WM, Tiemeier H, et al. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. *Aging Cell.* 2011; 10:686–698.
13. Deelen J, Beekman M, Uh H-W, Broer L, Ayers KL, Tan Q, Kamatani Y, Bennet AM, Tamm R, Trompet S, Guðbjartsson DF, Flachsbarth F, Rose G, et al. Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age. *Hum Mol Genet.* 2014; 23:4420–4432.
14. Broer L, Buchman AS, Deelen J, Evans DS, Faul JD, Lunetta KL, Sebastiani P, Smith J a, Smith A V, Tanaka T, Yu L, Arnold AM, Aspelund T, et al. GWAS of Longevity in CHARGE Consortium Confirms APOE and FOXO3 Candidacy. *J Gerontol A Biol Sci Med Sci.* 2014:1–9.
15. Beekman M, Nederstigt C, Suchiman HED, Kremer D, van der Breggen R, Lakenberg N, Alemayehu WG, de Craen AJM, Westendorp RGJ, Boomsma DI, de Geus EJC, Houwing-Duistermaat JJ, Heijmans BT, et al. Genome-wide association study (GWAS)-identified disease risk alleles do not compromise human longevity. *Proc Natl Acad Sci U S A.* 2010; 107:18046–18049.
16. Stevenson M, Bae H, Schupf N, Andersen S, Zhang Q, Perls T, Sebastiani P. Burden of disease variants in participants of the Long Life Family Study. *Aging (Albany NY).* 2015; 7:123–132.
17. Dutta A, Henley W, Robine JM, Langa KM, Wallace RB, Melzer D. Longer lived parents: Protective associations with cancer incidence and overall mortality. *Journals Gerontol - Ser A Biol Sci Med Sci.* 2013; 68:1409–1418.
18. Tan Q, Zhao JH, Li S, Kruse TA, Christensen K. Power assessment for genetic association study of human longevity using offspring of long-lived subjects. *Eur J Epidemiol.* 2010; 25:501–506.
19. Sierra F, Kohanski R. *Advances in Geroscience.* 2016.
20. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics.* 2010; 26:2336–2337.
21. Furberg H, Kim Y, Dackor J, Boerwinkle E, Franceschini N, Ardisson D, Bernardinelli L, Mannucci PM, Mauri F, Merlini PA, Absher D, Assimes TL, Fortmann SP, et al. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet.* 2010; 42:441–447.
22. Rebhan M, Chalifa-Caspi V, Prilusky J, Lancet D. GeneCards: integrating information about genes, proteins and diseases. *Trends Genet.* 1997; 13:163.
23. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, et al. Geroscience: Linking Aging to Chronic Disease. *Cell.* 2014; 159:709–713.
24. Dixon JB, Egger GJ, Finkelstein EA, Kral JG, Lambert GW. ‘Obesity paradox’ misunderstands the biology of optimal weight throughout the life cycle. *Int J Obes (Lond).* 2015; 39:82–84.
25. Mooijaart SP, van Heemst D, Noordam R, Rozing MP, Wijnsman C a, de Craen AJM, Westendorp RGJ, Beekman M, Slagboom PE. Polymorphisms associated with type 2 diabetes in familial longevity: The Leiden Longevity Study. *Aging (Albany, NY Online).* 2011; 3:55–62.
26. Yu C-E, Seltman H, Peskind ER, Galloway N, Zhou PX, Rosenthal E, Wijnsman EM, Tsuang DW, Devlin B, Schellenberg GD. Comprehensive analysis of APOE and selected proximate markers for late-onset Alzheimer’s disease: patterns of linkage disequilibrium and disease/marker association. *Genomics.* 2007; 89:655–665.
27. Lindahl-Jacobsen R, Tan Q, Mengel-From J, Christensen K, Nebel A, Christiansen L. Effects of the APOE ε2 allele on mortality and cognitive function in the oldest old. *J Gerontol A Biol Sci Med Sci.* 2013; 68:389–394.
28. Fortney K, Dobriban E, Garagnani P, Pirazzini C, Monti D, Mari D, Atzmon G, Barzilai N, Franceschi C, Owen AB, Kim SK. Genome-Wide Scan Informed by Age-Related Disease Identifies Loci for Exceptional Human Longevity. *PLOS Genet.* 2015; 11:e1005728.
29. Hunt KA, Zhernakova A, Turner G, Heap GAR, Franke L, Bruinenberg M, Romanos J, Dinesen LC, Ryan AW, Panesar D, Gwilliam R, Takeuchi F, McLaren WM, et al. Newly identified genetic risk variants for celiac disease related to the immune response. *Nat Genet.* 2008; 40:395–402.
30. Huan T et al. Integrative network analysis reveals molecular mechanisms of blood pressure regulation. *Mol Syst Biol.* 2015; 11:799.
31. Postmus I, Deelen J, Sedaghat S, Trompet S, de Craen a. J, Heijmans BT, Franco OH, Hofman a., Dehghan a., Slagboom PE, Westendorp RG, Jukema JW. LDL cholesterol still a problem in old age? A Mendelian randomization study. *Int J Epidemiol.* 2015; 73:604–612.
32. Atzmon G, Rincon M, Schechter CB, Shuldiner AR, Lipton RB, Bergman A, Barzilai N. Lipoprotein Genotype and Conserved Pathway for Exceptional Longevity in Humans. *PLoS Biol.* 2006; 4:e113.
33. Allen N, Sudlow C, Downey P, Peakman T, Danesh J, Elliott P, Gallacher J, Green J, Matthews P, Pell J, Sprosen T, Collins R. UK Biobank: Current status and what it means for epidemiology. *Heal Policy Technol.* 2015; 1:123–126.
34. Manolio TA, Collins R. Enhancing the feasibility of large cohort studies. *JAMA.* 2010; 304:2290–2291.
35. Cheung SLK, Robine J. Increase in common longevity and the compression of mortality: the case of Japan. *Popul Stud (NY).* 2007; 61:85–97.
36. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, Hottenga JJ, Fischer K, Esko T, Surakka I, Broer L, Nyholt DR, Leach IM, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet.* 2013; 45:422–427.
37. Global Lipids Genetics Consortium, Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet.* 2013; 45:1274–1283.
38. UK Biobank. UK Biobank genotype collections and QC on 150k participants, April 2014; http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/UKBiobank_genotyping_QC_documentation-web.pdf.
39. Abraham G, Inouye M. Fast principal component analysis of large-scale genome-wide data. *PLoS One.* 2014; 9:e93766.
40. Gauderman W, Morrison J. QUANTO 1.1: A computer

program for power and sample size calculations for genetic-epidemiology studies 2006.

41. Loh P-R, Tucker G, Bulik-Sullivan BK, Vilhjálmsson BJ, Finucane HK, Salem RM, Chasman DI, Ridker PM, Neale BM, Berger B, Patterson N, Price AL. Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat Genet.* 2015; 47:284–290.

42. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575.

43. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol.* 1979; 110:281–290.

44. Loh P-R, Bhatia G, Gusev A, Finucane HK, Bulik-Sullivan BK, Pollack SJ, Schizophrenia Working Group of the Psychiatric Genomics Consortium, de Candia TR, Lee SH, Wray NR, Kendler KS, O'Donovan MC, Neale BM, et al. Contrasting genetic architectures of schizophrenia and other complex diseases using fast variance-components analysis. *Nat Genet.* 2015; 47:1385–1392.

SUPPLEMENTARY METHODS

Selection of variants for GRS

Body mass index. We selected 69 of 76 common genetic variants associated with BMI at genome wide significance in the GIANT consortium studies of up to 339,224 individuals [1]. We limited the BMI SNPs to those that were associated with BMI in the analysis of all European ancestry individuals and did not include those that only reached genome-wide levels of statistical confidence in one-sex only, or one-strata only. Variants were also excluded if classified as a secondary signal within a locus. [Three SNPs were excluded due to potential pleiotropy (rs11030104, rs13107325, rs3888190)], 3 SNPs were not in Hardy-Weinberg Equilibrium (HWE) (rs17001654, rs2075650, rs9925964) and one SNP was unavailable (rs2033529) (Supplementary Table 1).

Coronary artery disease. Of 44 genetic variants associated ($p < 5 \times 10^{-8}$) with Coronary Artery Disease (CAD) in the recent meta-analysis of up to 185,000 cases and controls [2] one was excluded based on $R^2 > 0.1$ with another SNP (< 1 Mb distance) in the GRS, and one other was included as it was not available in the data; leaving 42 eligible SNPs to include (Supplementary Table 1).

Lipids: LDL, HDL and TG. The most recent Global Lipids Consortium meta-analysis [3] ($n = 188,577$) reported the following numbers of variants associated ($p < 5 \times 10^{-8}$) with: LDL (55), HDL (67), and TG (37). Three LDL SNPs were not in HWE (rs1800562, rs2902940, rs1250229) so were excluded. No further exclusions were required. Therefore 52 LDL SNPs, 67 HDL SNPs and 37 TG SNPs were included in the respective GRS' (Supplementary Table 1).

Coronary artery disease – independent of lipids. Of the 42 SNPs included in the CAD GRS we excluded 9 where the SNP (or an exact proxy) was present in the LDL meta-analysis and associated with LDL $p < 0.05$, an additional 4 SNPs that were associated $p < 0.05$ with HDL, and 6 SNPs for which no proxies in the lipid GWAS could be identified, to create a “CAD no lipids” GRS including 23 SNPs associated with CAD but not LDL, HDL or TG (Supplementary Table 1).

Systolic blood pressure. We generated GRS for systolic blood pressure (SBP) only, due to the substantial overlap between the SNPs identified for SBP and diastolic blood pressure, based on the results of a meta-analysis on up to 200,000 participants of European

ancestry [4]. All 26 SNPs for SBP were present in the UK BioBank imputation data with quality > 0.95 , and all HWE p -values $> 1 \times 10^{-6}$ (Supplementary Table 1).

Stroke. In 2014 a GWAS of 17,970 ischemic stroke cases reported 4 loci associated with stroke (rs17696736, rs2023938, rs12646447, rs10744777) [5]. All four were present in the imputed Biobank genetics data with quality > 0.95 and HWE p -values $> 1 \times 10^{-6}$ (Supplementary Table 1).

Alzheimer's disease. Results from a meta-analysis including 4,018 Alzheimer's Disease (AD) cases showed 7 loci significantly associated with late-onset AD [6]. All 7 were used to create the GRS in this study (imputation quality = 1 for all SNPs, HWE $p > 1 \times 10^{-6}$) (Supplementary Table 1).

Breast Cancer. The most recent breast cancer GWAS meta-analysis found 41 new loci, bringing the total to 66 [7]. One SNP (rs614367) was excluded due to HWE $p < 1 \times 10^{-6}$, leaving 65 for the GRS (Supplementary Table 1).

Prostate cancer. The most recent GWAS meta-analysis identified 23 new loci for prostate cancer (PC), bringing the total to 87 [8]. We used the results from participants on European descent (35,093 cases, 34,599 controls) to generate the risk score. All SNPs have imputation quality > 0.95 , however 2 SNPs (rs1775148 and rs1983891) were excluded due to HWE $p < 1 \times 10^{-6}$, meaning 85 SNPs were included in the GRS (Supplementary Table 1).

Inflammatory bowel disease. Inflammatory Bowel Disease (IBD) is commonly defined as presence of either Crohn's Disease (CD) or Ulcerative Colitis (UC), and a recent GWAS meta-analysis found significant overlap between CD and UC, but also some genetic divergence [9]. In total 232 unique SNPs are reported with either CD, UC or IBD in the 86,640 individuals of European descent in the study, 142 with CD, 89 with UC, and 159 with IBD. We generated three GRS based on these lists: for IBD 3 SNPs were excluded due to HWE $p < 1 \times 10^{-6}$ (rs4703855, rs224090, rs17622378), for CD the same 3 SNPs were excluded, and for UC 2 SNPs were excluded due to HWE $p < 1 \times 10^{-6}$ (rs17622378 and rs17771967 – the latter is unique to the UC list of significantly associated SNPs). Therefore the following number of SNPs were included: 156 for IBC, 139 for CD and 87 for UC (Supplementary Table 1).

Forced vital capacity. In an analysis of 85,170 participants for Forced Vital Capacity (FVC) 6 loci were significant in the combined meta-analysis [10]. All

were imputed in UK BioBank with high quality (>0.95) and with HWE $p > 1 \times 10^{-6}$. Therefore all 6 SNPs were included in the FVC GRS (Supplementary Table 1).

Age at menopause. Fifty four common SNPs were identified or confirmed in the most recent GWAS of age-at-menopause, in addition to 3 low-frequency (MAF $>0.5\%$ and $<5\%$) coding variants identified by exome array [11]. One “exome SNP” (rs148126992) was excluded because it is not independent of rs75770066, which is more significant in the meta-analysis. Four of the common SNPs (rs6484478, rs3741604, rs1183272, rs7397861) were excluded because their association with age-at-menopause was only significant ($p < 5 \times 10^{-8}$) in joint analyses for regions containing more than one SNP. Therefore 52 SNPs were included in the GRS. Greater values of age-at-menopause GRS equate to earlier menopausal age.

Telomere length. Seven loci were identified to be associated with Telomere Length (TL) in a recent GWAS meta-analysis [12]. All 7 were imputed with high quality and no significant deviation from HWE was observed, therefore all 7 contributed to the GRS. Greater values of TL GRS equate to longer telomeres.

Type-1 diabetes. In total 29 SNPs were used to create the GRS for Type-1 Diabetes (T1D), using the methods as described in [13] (Supplementary Table 1). Two SNPs (rs2187668, rs7454108) were used to determine an individual’s haplotype combination (DR3/DR4-DQ8) with each haplotype combination having an associated effect size. 27 out of 30 non-HLA SNPs were utilized in addition to the HLA haplotype-tagging SNPs (3 were excluded due to HWE $p < 5 \times 10^{-6}$).

Type-2 diabetes. Of 65 loci confirmed to be associated with Type-2 Diabetes (T2D) in a recent GWAS meta-analysis (34,840 cases) [14] 10 were excluded due to non-specific effects (e.g. primary effect of rs12970134 is on BMI, therefore excluded here) and one was substituted for a proxy (rs11651052 substituted for rs4430796). Therefore 55 SNPs are included in the GRS (see Supplementary Table 1 for details).

SUPPLEMENTARY REFERENCES

1. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015; 518:197–206.
2. The CARDIoGRAMplusC4D Consortium. A comprehensive 1000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat. Genet.* 2015; 47.
3. Global Lipids Genetics Consortium, Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, et al. Discovery and refinement of loci associated with lipid levels. *Nat. Genet.* 2013;

45:1274–1283.

4. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. Nature Publishing Group; 2011; 478:103–109.
5. Kilarski LL, Achterberg S, Devan WJ, Traylor M, Malik R, Lindgren A, et al. Meta-analysis in more than 17,900 cases of ischemic stroke reveals a novel association at 12q24.12. *Neurology*. 2014; 83:678–685.
6. Kamboh MI, Demirci FY, Wang X, Minster RL, Carrasquillo MM, Pankratz VS, et al. Genome-wide association study of Alzheimer’s disease. *Transl. Psychiatry*. 2012; 1–7.
7. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat. Genet.* 2013; 45:353–61, 361e1–2.
8. Al Olama AA, Kote-Jarai Z, Berndt SI, Conti D V, Schumacher F, Han Y, et al. A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. *Nat. Genet.* 2014; 46:1103–1109.
9. Liu JZ, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.* 2015.
10. Loth DW, Artigas MS, Gharib SA, Wain L V, Franceschini N, Koch B, et al. Genome-wide association analysis identifies six new loci associated with forced vital capacity. *Nat. Genet.* 2014; 46:669–677.
11. Day FR, Ruth KS, Thompson DJ, Lunetta KL, Pervjakova N, Chasman DI, et al. Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair. *Nat. Genet.* 2015; 47.
12. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat. Genet.* 2013; 45:422–427.
13. Oram RA, Patel K, Hill A, Shields B, McDonald TJ, Jones A, et al. A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. *Diabetes Care*. 2015; 1–8.
14. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segrè A V, Steinthorsdottir V, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat. Genet.* 2012; 44:981–990.

SUPPLEMENTARY TABLES

Please browse Full text version of this manuscript to see Supplementary Tables:

Supplementary Table 1. Lists the genetic variants included for each genetic risk score.

Supplementary Table 2. Includes the 1,000 most-associated variants from each of the four GWAS performed.

Supplementary Table 3. Includes the full results for all associations between the genetic risk scores and the four parent's age-at-death phenotypes.

Supplementary Table 4. The results from the four GWAS performed for a specific set of variants with *a priori* hypotheses regarding human longevity.

SUPPLEMENTARY FIGURES

Please browse Full text version of this manuscript to see Supplementary Figures:

Supplementary Figure 1. Flow-chart demonstrating the derivation (and number of participants included) for each of the four phenotypes/analyses utilized in the analyses.

Supplementary Figure 2. QQ plot and Manhattan plot for the GWAS of father's age at death.

Supplementary Figure 3. QQ plot and Manhattan plot for the GWAS of mother's age at death.

Supplementary Figure 4. QQ plot and Manhattan plot for the GWAS of combined parents' age at death.

Supplementary Figure 5. QQ plot and Manhattan plot for the GWAS of extreme longevity (at least one parent lived to the top 1% of the age at death distribution in the Biobank respondents).

Supplementary Figure 6. LocusZoom plot shows the chromosomal locations of variants within 250kbp of rs1051730 (x-axis), and the degree of association with father's age at death (y-axis) in the GWAS. The colors reflect the degree of linkage disequilibrium (red = high) with rs1051730 (purple).

Supplementary Figure 7. LocusZoom plot shows the chromosomal locations of variants within 250kbp of rs62227724 (x-axis), and the degree of association with mother's age at death (y-axis) in the GWAS. The colors

reflect the degree of linkage disequilibrium (red = high) with rs62227724 (purple).

Supplementary Figure 8. LocusZoom plot shows the chromosomal locations of variants within 250kbp of rs75824829 (x-axis), and the degree of association with extreme longevity (y-axis) in the GWAS (at least one parent lived to the top 1% of age at death distribution in UK Biobank respondents). The colors reflect the degree of linkage disequilibrium (red = high) with rs75824829 (purple).

Supplementary Figure 9. LocusZoom plot shows the chromosomal locations of variants within 250kbp of rs79109928 (x-axis), and the degree of association with extreme longevity (y-axis) in the GWAS (at least one parent lived to the top 1% of age at death distribution in UK Biobank respondents). The colors reflect the degree of linkage disequilibrium (red = high) with rs79109928 (purple).

LocusZoom utilizes information from the Nov 2014. Release of the 1000 genomes panel, which does not appear to include variant rs528161076, the most-associated variant in the GWAS. We searched for proxies for this variant in the UK Biobank respondents and found that rs79109928 (located 1323 bases from rs528161076) is in high linkage disequilibrium ($R^2=0.943$); we therefore created the LocusZoom plot focused on rs79109928 and have included a "star" symbol to represent the location of rs528161076, and the degree of association with extreme longevity.

Supplementary Figure 10. Forest plot showing the associations between the GRS for CAD, LDL, HDL and AD with and without the APOE variant rs4420638.