Rare genetic coding variants associated with human longevity and protection against age-related diseases

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Extreme longevity in humans has a strong genetic component, but whether this involves genetic variation in the same longevity pathways as found in model organisms is unclear. Using whole-exome sequences of a large cohort of Ashkenazi Jewish centenarians to examine enrichment for rare coding variants, we found most longevity-associated rare coding variants converge upon conserved insulin/insulin-like growth factor 1 signaling and AMP-activating protein kinase signaling pathways. Centenarians have a number of pathogenic rare coding variants similar to control individuals, suggesting that rare variants detected in the conserved longevity pathways are protective against age-related pathology. Indeed, we detected a pro-longevity effect of rare coding variants in the Wnt signaling pathway on individuals harboring the known common risk allele APOE4. The genetic component of extreme human longevity constitutes, at least in part, rare coding variants in pathways that protect against aging, including those that control longevity in model organisms.

Species-specific lifespan is limited by aging, a multifactorial process accompanied by a general decline in tissue function and increased risk for many diseases. Rather than a passive, entropic process of deterioration, aging is subject to active modulation by signaling pathways and transcription factors conserved across species. In model organisms, single-gene mutations have been demonstrated to affect lifespan. For example, at the extreme end, the lifespan of nematode worms can be increased up to nearly tenfold by mutations in genes involved in insulin/insulin-like growth factor 1 signaling (IIS). Even in more complicated organisms, however, such as flies and mice, lifespan can be extended up to 50% by mutations affecting the same pathways or other pathways involved in growth, metabolism and nutrient sensing, such as the mechanistic target of rapamycin (mTOR) and AMP-activating protein kinase (AMPK). On the basis of homology, it is widely hypothesized that these conserved signaling pathways are similarly involved in human aging and longevity.

In humans, lifespan is a complex trait affected by multiple factors that vary considerably within human populations. While nongenetic factors, including diet, physical activity, health habits and psychosocial factors, are important, lifespan clearly has a genetic component as suggested by human population-based studies. At increasingly older ages, especially beyond 100 years, this genetic component becomes exceedingly strong. As a highly complex trait, the genetic underpinnings of human lifespan probably encompass different types of genetic variants and epistasis across the allele frequency spectrum. Common variants associated with human survival have been extensively searched for in many recent genome-wide association studies (GWAS), using a variety of trait definitions and study designs. Together, these studies have identified more than 50 longevity-associated genetic loci of genome-wide significance, among which only a few, especially APOE, were replicated by multiple studies. On the other hand, several previous studies have detected an association of human longevity with variants in several aging genes—including insulin signaling genes and FOXO3 (refs. 13,13)—using candidate gene approaches. Most of these longevity-associated SNPs have small effect sizes, and currently common variants collectively explain only a very small proportion of human longevity.
of heritability for human longevity. As several recent studies suggest, rare variants probably account for at least some of the ‘missing’ heritability20–22.

Here we examined rare coding variants in a cohort of 515 Ashkenazi Jewish centenarians by whole-exome sequencing (WES) and tested for enrichment using a case-control design. The exceptional longevity of this cohort and their homogeneous genetic background provided us with increased power to detect causal rare variants23. As controls we used 496 Ashkenazi Jewish individuals, mostly from the same households as the centenarians and between age ~70 and 95 years with no parental history of extreme longevity (neither parent survived beyond 95 years of age) (Table 1 and Supplementary Table 1).

### Results

**Longevity genes and pathways implicated by rare variants.** Using a joint genotyping procedure and stringent quality control metrics, we identified 130,297 rare coding variants, including 126,405 single-nucleotide polymorphisms (SNPs) and 3,892 indels with minor allele frequencies <0.01 and missing rates <0.1 in 17,561 genes in centenarians and controls. Of all SNPs, a total of 45,493 were found to be synonymous. The remaining 84,804 nonsynonymous SNPs and all indels include 75,567 missense variants, >3,500 loss-of-function variants (1,755 frameshift, 1,736 stop-gain and 79 stop-loss variants) and other rare variants with multiple functional annotations. We did not exclude synonymous rare variants from our analysis because not all are functionally silent24. At the whole-exome level we found no significant difference in the number of rare coding variants between centenarians and controls ($P = 0.243$, logistic regression including gender and the top ten multidimensional scaling (MDS) components as covariates).

We next examined the association of rare variants with longevity at the variant or gene level. At the variant level, we applied the Firth logistic regression for rare variant association tests to examine the association between the minor allele count of each rare coding variant and longevity status. The variant with the strongest association signal was rs2229426 in fatty acid synthase (FASN) ($P = 6.23 \times 10^{-7}$) (Supplementary Table 2). At the gene level, we applied two complementary region-based association tests—the 'burden test of rare variants' and the sequence kernel association test (SKAT)—to discover rare variants through statistically significant genetic modeling. The burden test searches for a significant excess of rare alleles in longevity cases or controls, while SKAT implements a variance component test to detect the effects of rare variants on longevity even if they have opposite directions. Chloride voltage-gated channel 6 (CLCN6) presented the strongest variant association with longevity (burden test, $P = 3.45 \times 10^{-8}$ and $1.03 \times 10^{-7}$ as the lowest and combined $P$ value, respectively) (Supplementary Table 3). Although these top associations at the variant or gene level did not reach genome-wise significance after multiple-test correction, quantile-quantile (QQ) plots of association signals showed upward deviation in the tails—the lowest $P$ values smaller than expected from uniform distribution (0, 1)—for several groups of rare variants such as functional rare variants (combined annotation-dependent depletion (CADD) score ≥20) and functional but recessive benign rare variants (CADD ≥20 and PrimateAI score <0.5) (see the variant masking in Methods for variant groups and their interpretation; Fig. 1a,b, Supplementary Figs 1 and 2 and Supplementary Table 4). These rare variants include several genes known to be related to aging, such as FASN25 and the DNA repair gene BLM RecQ-like helicase (BLM)26.

The extreme rarity of centenarians in human populations essentially constrains the possibility of performing the large studies necessary to discover rare variants through statistically significant genetic associations with longevity. Instead of a candidate gene approach, we used integrated gene signal processing (IGSP) to prioritize genes based on the longevity association of rare variants in an unbiased manner through data integration20. About 94% of human protein-coding genes were in the functional linkage network used by IGSP, and only half of them also had knockout phenotype data for their mouse homologs. To include most genes in our analysis, we opted for a network integration rather than a full one, which needs both gene network and mouse phenotype data. Individual genes were scored by joint analysis of the longevity association of genes implicated by rare variants in a gene functional linkage network (predicted based on independent genomic high-throughput data)27, which implicitly incorporates information on gene–gene functional similarity. Data simulation showed that such integrated scoring greatly increases the prioritization power and effectively uncovers risk genes with marginal association signals20. The negative-control evaluation showed that ~100 top-ranked genes had higher IGSP scores when scored by real data than by randomized data ($P = 0.037$) (Supplementary Fig. 3 and Supplementary Table 5), which suggests a clustering of longevity-associated genes implicated by rare variants in the gene network captured by a network integration in gene scoring28. Subsequent pathway enrichment analysis showed that these predicted longevity genes are significantly enriched in insulin signaling (false discovery rate (FDR) = 0.00879) and mTOR signaling (FDR = 0.0129) (Fig. 1c and Supplementary Fig. 4). Some predicted longevity genes have an indirect connection to insulin signaling as they are in the pathway of signaling by the insulin receptor (for example, PSMB9). Interestingly, many of the putative longevity genes carry a burden of rare variants in centenarians, among which potential protective rare variants were also found in previous studies such as those on ABCA1 (ref. 29) and PLCG2 (ref. 30) (Supplementary Table 5).

To further increase power, the longevity association of rare coding variants can be studied at the pathway level. Since aging is characterized by evolutionarily conserved, parallel and interacting mechanistic hallmarks, we next analyzed rare variants collectively in 20 pathways of all nine aging hallmarks (Supplementary Table 6). Functional but recessively benign rare variants in insulin signaling (SKAT, $P = 5.57 \times 10^{-6}$, FDR = 0.012) and AMPK signaling (SKAT, $P = 1.59 \times 10^{-4}$, FDR = 0.017) pathways were found to be significantly associated with extreme longevity (Fig. 1d and Supplementary Tables 7 and 8) after multiple testing correction that took into account the total numbers of pathways, tests and variant masks.
When studying genetic variants in association studies, it is important to validate the results by replicating any observed association signals in unrelated cohorts. Our approach followed the sequence-based replication strategy, which is more powerful than the variant-based strategy that analyzes only rare variants uncovers in the discovery cohort. Specifically, we examined three replication cohorts for the longevity association of rare coding variants in the insulin and AMPK signaling pathways (Supplementary Table 9): a German longevity cohort of 1,265 centenarians (mean age 99 years) and 4,195 blood donors (mean age 35 years) as controls; a UK Biobank longevity cohort of 104 participants with at least one long-lived parent (lifespan ≥100 years) and 23,405 participants with parents of usual survival (lifespan <95 years); and an Alzheimer’s disease (AD) sequencing project (ADSP) longevity cohort of 1,121 non-AD individuals aged ≥90 years and 38 non-AD individuals aged <75 years.

In the German longevity cohort, we detected a significant longevity association of functional but recessive benign ultra-rare variants (alternative allele frequency (AAF) <0.05% among non-Finnish Europeans in gnomAD) in insulin signaling (SKAT, \( P = 4.41 \times 10^{-4} \), FDR = 0.018) after appropriate multiple testing correction (Extended Data Fig. 1a). In the UK Biobank longevity cohort, we identified significant longevity associations of functional rare variants in insulin signaling (SKAT, \( P = 9.64 \times 10^{-4} \), FDR = 3.87 \times 10^{-2} \) and functional but recessive benign ultra-rare variants in AMPK signaling (SKAT, \( P = 2.08 \times 10^{-5} \), FDR = 0.041) pathways (Extended Data Fig. 2a). In the ADSP longevity cohort, we identified significant longevity associations of recessive pathogenic rare variants in insulin signaling (burden test, \( P = 8.98 \times 10^{-5} \), FDR = 3.6 \times 10^{-3} \); direction on controls) (Extended Data Fig. 3).

Next, we focused on identifying rare variants associated with human age-related disease. A genetic relationship between extreme human longevity and disease is supported by multiple observations in independent studies of a genetic association between extreme human longevity and APOE, a locus causally related to both cardiovascular and neurodegenerative disease. Here we hypothesized that rare genetic variants associated with human longevity can exert their beneficial effects, at least in part, by protecting against chronic disease. Hence, we examined the rare coding variants in the 20 aging hallmark pathways in more refined subgroups of our cohort based on their APOE haplotype status, and analyzed separately the longevity subcohorts of APOE4 carriers and noncarriers (hereinafter APOE4- and APOE4+, respectively) to identify longevity-associated pathways in these two distinct genetic backgrounds. Among APOE4-, functional but recessively benign rare variants in both insulin and AMPK signaling pathways were again found to be significantly associated with longevity (SKAT, \( P = 6.21 \times 10^{-6} \) and 7.9 \times 10^{-3} , FDR = 2.63 \times 10^{-3} and 0.013, respectively) (Extended Data Fig. 4 and Supplementary Tables 10 and 11). Interestingly, among APOE4+ we detected a significant association between longevity and 152 functional rare variants in Wnt signaling genes after multiple testing correction using both the burden test and SKAT (burden test, \( P = 9.16 \times 10^{-5} \), FDR = 0.013; SKAT, \( P = 3.40 \times 10^{-4} \), FDR = 0.036) (Extended Data Fig. 4 and Supplementary Tables 12 and 13). The direction of association suggests that these rare variants are enriched for protective variants among centenarian APOE4+ in our cohort (Supplementary Table 14). Indeed, only six of them were predicted as highly pathogenic rare variants (PrimateAI score ≥0.9) and they are not enriched, either individually or collectively, among APOE4+ centenarians. The Wnt association was replicated in the UK Biobank longevity cohort with a significant longevity association of functional rare variants in Wnt signaling pathway among APOE4+ (SKAT, \( P = 1.79 \times 10^{-10} \), FDR = 2.14 \times 10^{-4} \) (Extended Data Fig. 2b and Supplementary Table 9). We did not detect significant longevity association signals from rare variants in the Wnt signaling pathway in either of the APOE4 stratified German longevity subcohorts (Extended Data Fig. 1b).

We examined further the protective effect of functional rare variants in Wnt signaling genes on individual human lifespan in our lifespan cohort of 553 individuals with verifiable age at death. Starting with the full linear model of lifespan that included gender, APOE4 status, the alternative allele count of protective rare variants in Wnt signaling and all two- and three-way interaction terms among them, we identified a statistically significant interaction, the only one, between APOE4 status and allele count in Wnt signaling (\( P = 1.13 \times 10^{-8} \)) (Supplementary Table 15). Because APOE4 status is determined mainly by rs429358, a common variant (minor allele frequency (MAF) = 0.14) associated with aging and age-related diseases, the lifespan analysis result indicates the existence of epistasis between rare variants and aging-associated common variants in the genetic architecture of human aging. We then analyzed the relationship between individual lifespan and the alternative allele count of protective rare variants in Wnt signaling in subcohorts, stratified by the status of both longevity and APOE4 (Fig. 2a). Among centenarians, allele count in Wnt signaling has no effect on lifespan regardless of APOE4 status. Among noncentenarians there was a significant positive correlation between the burden of Wnt rare variants and lifespan among APOE4+ (\( r = 0.406, P = 8.39 \times 10^{-3} \), FDR = 0.026; Fig. 2a, middle) compared with noncarriers. The relationship between APOE4 status and allele count in Wnt signaling can also be more readily appreciated by comparing the average lifespan of subcohorts stratified based on both APOE and Wnt signaling. Among APOE4+, the median difference in lifespan was >9 years (\( P = 2.38 \times 10^{-3} \)) between individuals with low and high allele counts in Wnt signaling (Fig. 2b). The negative effect of APOE4 on lifespan became weaker among individuals with a high burden of potentially protective Wnt rare variants (Fig. 2c). Interestingly, the aforementioned 152 rare variants in Wnt signaling genes are associated with the disease status of individuals in the ADSP (SKAT, \( P = 4.82 \times 10^{-8} \)). Finally, using the same framework, we analyzed lifespans of centenarians and noncentenarians separately and demonstrated a similar protective effect of the 152 rare variants in Wnt signaling genes (Supplementary Table 14) on lifespan among noncentenarian APOE4+ (Supplementary Table 9 and Extended Data Figs. 5 and 6).
Longevity and common polygenic risk of age-related diseases. The phenotypic outcome of individuals that carry rare variants of large effects can also be influenced by the background of common polygenic variation. To assess how rare variants may interact with the genetic background of common variants to affect human aging, we specifically examined in our longevity cohort common variants associated with seven age-related diseases: AD, coronary artery disease (CAD), type 2 diabetes (T2D), stroke, breast cancer, prostate cancer and pancreatic cancer. This analyzed cohort consists of 479 centenarians and 431 controls with both WES and SNP array data available (Table 1 and Supplementary Table 1). We calculated polygenic risk scores (PRS) of individuals for these diseases using summary statistics associated with rare coding variants (CADD ≥ 20) and synonymous rare variants (CADD ≥ 20).
from their corresponding GWAS (Methods). Empirical P-values provided by PRSice-2 that account for overfitting indicated significant genetic overlap between longevity and each of AD, CAD and T2D (Fig. 3, Extended Data Fig. 7 and Supplementary Table 16), which was further supported by the significant results of cross-validation (Supplementary Table 17). PRS for AD, CAD and T2D explained 1.93% (P=0.0019), 1.32% (P=0.013) and 1.29% (P=0.015) variance of longevity status, respectively. Measured by PRS, centenarians
Fig. 3 | Common polygenic risk of age-related diseases. a, Common polygenic risk for seven different age-related diseases of subjects were calculated using PRS of the corresponding disease. Nagelkerke’s $R^2$ is based on correlation between disease PRS and centenarian status. Bar color denotes the statistical significance of $R^2$ after adjusting for MDS1–10 and gender (except breast cancer and prostate cancer, which were tested with females and males, respectively) as covariates. Statistical significance is based on the permutation $P$ values of using PRSice-2. For AD and CAD, middle bars show the results of PRS analyses excluding SNPs within 1 megabase pair (Mb) of APOE haplotype SNPs rs7429358 and rs7412. Bottom bars (for AD, CAD, breast cancer and prostate cancer) show the results of PRS analyses using extreme-longevity phenotypes (cases and controls aged ≥100 and <80 years, respectively) as covariates. Statistical significance is based on the permutation $P$ values for the best prediction in Nagelkerke’s $R^2$ plot on the left, which were calculated based on logistic regression and permutation testing in PRSice-2, respectively. Double and triple asterisks denote adjusted $P$ values smaller than 0.01 and 0.001, respectively.

We applied an ‘extreme-longevity phenotyping’ strategy and found that the variance explained by PRS for AD and CAD increased by almost fourfold to ~4–7% for people aged ≥100 and <80 years (Fig. 3, Supplementary Fig. 5 and Supplementary Table 16). PRS for T2D showed no such gender difference (Supplementary Fig. 6).

tend to have reduced genetic susceptibility not only to AD and CAD (Bonferroni–Holm $P^* = 0.0067$ and $0.039$, respectively), which were previously found to be associated with healthy aging\(^5\), but also T2D ($P^* = 0.039$). The predictive power of PRS for AD was mainly driven by the APOE haplotype defined by SNPs rs7412 and rs429358 (Fig. 3b,c), but this is not the case for CAD (Extended Data Fig. 7b)\(^5\). To further examine genetic overlap between longevity and diseases, we applied an ‘extreme-longevity phenotyping’ strategy and found that the variance explained by PRS for AD and CAD increased by almost fourfold to ~4–7% for people aged ≥100 and <80 years (Fig. 3, Supplementary Fig. 5 and Supplementary Table 16). PRS for T2D showed a stronger association with longevity status among males than females in our cohort; PRS for AD and CAD, however, showed no such gender difference (Supplementary Fig. 6).
Pathogenic rare variants and longevity. Since the genetic component of extreme longevity can be explained, at least in part, by a reduced burden of pathogenic variants as compared with that of the general population, we compared the counts of predicted pathogenic rare coding variants (PrimateAI score ≥0.9). No significant difference between centenarians and controls was observed (P = 0.476, logistic regression including gender and the top ten MDS components as covariates; Fig. 4a). Using our lifespan cohort, we next investigated whether pathogenic rare coding variants affect lifespan, whether the effect depends on the common polygenic disease background and whether the effect is different between centenarians and controls. Consistent with the general observation, females also had significantly better survivorship than males in our lifespan cohort (P = 1.71 × 10⁻⁷; Extended Data Fig. 8), as did APOE4+ compared with APOE4− (P = 9.32 × 10⁻⁴; Extended Data Fig. 9). In our lifespan cohort, 853 pathogenic rare variants were identified. No correlation between the exome-wide burden of pathogenic rare variants and lifespan was observed among centenarians and non-centenarians together (the full lifespan cohort), or between either of them separately (Fig. 4b).
Human extreme longevity could be causally driven by a lack of genetic risk factors for chronic disease, by protective variants or by both. As measured by PRS, centenarians in our cohort tend to have reduced genetic susceptibility to AD, CAD and T2D among the seven age-related diseases that we examined (Fig. 3 and Supplementary Table 16). Using APOE4 status and PRS of CAD and T2D, we stratified our lifespan cohort according to their common genetic risk of AD, CAD and T2D—the three age-related diseases having significant genetic overlap with longevity in our cohort—and examined how the common polygenic disease risk background and pathogenic rare variants may together affect human lifespan. We first re-examined the effect of pathogenic rare variants on lifespan with an AD risk background based on APOE4 status, and found a weak negative correlation ($r = -0.184, P = 0.064$) among APOE4+. However, this relationship became significantly stronger ($r = -0.605, P = 2.85 \times 10^{-3}, FDR = 7.13 \times 10^{-7}$) when substantial genetic risk of both CAD and T2D was included (that is, APOE4+ with PRS for both diseases is higher than the respective median of the longevity cohort) (Fig. 4c and Supplementary Table 18). These results suggest interaction between pathogenic rare variants and disease-associated common variants. Such genetic interactions may affect the deleterious effect of pathogenic rare variants on human lifespan, a possibility that we formally investigated using a full linear model of lifespan including gender, APOE4 status, separate PRS of CAD and T2D, pathogenic rare variant counts and all two-way and higher-order interaction terms among them. The subsequent stepwise model selection identified multiple interactions, among which the most significant is a three-way interaction between pathogenic rare variant count and common polygenic disease risk of AD and T2D in our lifespan cohort ($P = 3.12 \times 10^{-4}$; Supplementary Table 15). Our analyses of stratified subcohorts showed that the negative effect of common polygenic disease risk on human lifespan can intensify under a high burden of pathogenic rare variants. For example, the presence of APOE4 reduced lifespan by ~1.5 years on average in our cohort. However, among individuals with at least seven pathogenic rare variants (median = 3), APOE4+ lived ~17 years fewer than noncarriers in general ($P = 2.77 \times 10^{-3}, FDR = 8.31 \times 10^{-4}$; Supplementary Fig. 7). To replicate this discovery of the relationship between pathogenic rare variants and lifespan, we first constructed a UK Biobank parental lifespan cohort (Methods), which consists of 20,823 unrelated (to first-degree kinship) participants with known parental age at death, and then examined the relationship between the exome-wide burden of pathogenic rare coding variants and parental lifespan in this cohort (Supplementary Fig. 8). We observed a negative correlation among APOE4+ ($r = -0.024, P = 0.044$; Supplementary Fig. 9 and Supplementary Table 9). The stepwise model selection procedure identified a significant interaction related to parental lifespan between APOE4 status and the exome-wide burden of pathogenic rare coding variants ($P = 5.48 \times 10^{-3}$).

Discussion
In summary, in this large-scale genetic study of rare coding variants and human longevity, our network-integrated analysis identified enrichment of longevity-associated rare coding variants in conserved aging pathways, and gene set association tests confirmed the longevity association of rare variants in the insulin and AMPK signaling pathways. These results suggest that rare variants in conserved aging pathways, important for aging of model organisms, also affect human lifespan and constitute a part of the genetic architecture of human longevity. As expected, based on the many species-specific characteristics of aging, this pattern is not completely identical between human and animal longevity. For example, we did not find any association of extreme longevity with variants in the mTOR pathway, which has been associated with longevity in model organisms including the mouse. On the other hand, we did find other pathways critical to human aging not yet identified in model organisms. For example, we demonstrated protective effects of rare variants in Wnt signaling on human lifespan. Interestingly, in the klotho-knockout mouse model of accelerated aging, continuous Wnt exposure triggered accelerated cellular senescence, implicating Wnt signaling in mammalian aging46. Finally, our results confirm previous reports that centenarians do not have a lower burden of pathogenic variants. Instead, from our present study, it appears that rare protective variants suppress the adverse effects of pathogenic variants on longevity.

To investigate whether the same conserved pathways are important to aging of both model organisms and humans, we could examine the effects of rare variants on lifespan-related traits. This is particularly challenging, however, due to a strong intrinsic stochasticity in aging processes: among isogenic Caenorhabditis elegans in a constant environment, lifespan of long-lived (age 1) mutants overlapped with that of wild-type controls47,48. Thus, the same genetic variants may have highly variable effects on lifespan among different individuals. While this stochasticity complicates the identification of longevity-associated variants in conserved aging pathways, the use of appropriate statistical tests and study cohorts can help overcome the challenge.

In this study we identified rare coding variants in aging pathways that affect human longevity. Future studies of their molecular functions could generate actionable biological insights on aging. In particular, uncovering the downstream pathways that mediate protective effects of rare variants found in Wnt signaling genes is imperative to translate this finding into therapeutic interventions against age-related diseases. Experiments with mouse cells suggest that APOE4 may inhibit Wnt signaling50. Dysregulation of Wnt signaling contributes to different types of age-related diseases such as cancer51, AD45 and cardiovascular disease52. Thus, protective rare variants in Wnt signaling genes could counteract the adverse effects of APOE4-induced Wnt inhibition on the progression of downstream age-related diseases and thus affect lifespan. While coding variants are more likely to reduce than to enhance the function of the protein product, conclusive confirmation and understanding of the functional effects at the molecular, cellular and organismal levels require experimental validation using functional assays and genome editing45.

Our study suggests that rare variants can have distinct effects on lifespan in different genetic backgrounds of age-related diseases (such as APOE4 status), underlying the difficulty in detecting and replicating the effects of rare variants on lifespan without considering other genetic factors. On the other hand, while common variants associated with age-related diseases are known to influence lifespan6, our finding of potential genetic interactions between common and rare variants in the context of the human lifespan provides insights into the mechanism of disease resilience as a part of the genetics of healthy aging among centenarians. How perturbation of conserved aging pathways contributes to human longevity and healthspan cannot be answered by genetics alone. However, while the molecular mechanisms of many conserved aging pathways have been widely studied in model organisms, our findings on rare variants—especially those from centenarians with high common polygenic risk of age-related diseases—can help translate those established longevity-regulating mechanisms in model organisms to therapeutic targets for healthy aging of humans.

A limitation of WES, as used in our present study, is the absence of rare, noncoding variants that have been implicated in aging of model organisms54–59 and that are thus of potential interest regarding human longevity. These include, for example, rare variants in noncoding RNAs or other regulatory elements relevant for tissue specificities, and variants in long tandem repeats connected to brain health and various neurological disorders. To identify the latter, long sequencing reads are required60.
Methods
Our WES study on the Einstein longevity cohorts complies with all relevant ethical regulations and was approved by the Institutional Review Board at Albert Einstein College of Medicine. Informed consent was obtained from participants, or from a proxy if the participant lacked decisional capacity. The WES studies of all three replication cohorts have informed consent from participants and were approved by the respective ethics committees or institutions: the Ethics Committee at Medical Faculty of Kiel University for the German longevity cohort; the Ethics Advisory Committee and the external ethics committees for the UK Biobank; and the ethics committees of the Broad Institute, Baylor College of Medicine’s Human Genome Sequencing Center and Albert Einstein College of Medicine since 1998. Cases (centenarians) were defined as individuals of age ≥95 years, while individuals of age <95 years without a parental history of longevity (neither parent survived beyond 95 years of age) were classified as controls. The centenarians’ dates of birth were confirmed by birth certificates or government-issued identification. Vital status and date of death, where applicable, were determined as of 3 April 2019, based on documentation of last contact with the study participant, reports from the next of kin and a search of publicly available databases. In the LGP and LonGenity cohorts, 555 and 508 individuals were classified as longevity cases (mean age 101 years) and controls (mean age 83 years), respectively. Mortality status was confirmed for 650 individuals, and these individuals were adopted as subjects for the lifespan analysis.

SNP array genotyping. SNP array genotyping was performed using Illumina Global Screening Array-24 v1.0 BeadChip with 642,824 markers, 7,201 of which could not be ‘lifted over’ to human genome assembly GRCh38 and were thus removed; a total of 2,026 samples were genotyped by SNP array. After removal of duplicates and samples not in our longevity studies, 635,623 variants in 1,830 samples were processed and analyzed (1,740 samples also have WES data). Quality control of array-based genotyped data was carried out using PLINK software (v.1.9)\(^1\). First, we checked the missing rate of SNPs and samples. SNPs and samples that missed >20% genotype calls were removed, and this missingness filtering was then repeated with a more stringent threshold of 2%. Individuals whose self-reported gender was different from that predicted based on sex chromosome heterozygosity were removed. SNPs whose genotype frequencies deviated from Hardy–Weinberg equilibrium with a \(p\) -test \(P < 1 \times 10^{-10}\) among controls, followed by \(P < 1 \times 10^{-15}\) among cases, were removed. Finally, samples whose heterozygosity deviated by more than three standard deviations from the mean were removed.

Exome sequencing and genotyping. Exome sequencing of 2,112 individuals in the LGP and LonGenity cohorts was performed at the Regeneron Genetics Center (RGC). Sample preparation and WES were performed using previously described methods (Supplementary Note). Variants in our centenarian cohort were called on human genome assembly GRCh38. For our rare variants analyses using both binary (cases versus controls) longevity and continuous lifespan data, only rare variants with missing rates \(<0.1\) in the corresponding study cohorts were analyzed; all samples in our study cohorts had a missing rate \(<0.1\) on rare variants that passed quality control (Supplementary Note).

Aggregation of SNP array and WES data. For PRS-related analyses, we used genotypic data aggregated from WES and SNP array (Extended Data Fig. 10a) for two reasons: (1) genotypes of common variants from the whole genome (not just the exome) need to be imputed (Genotype imputation) for PRS calculation; and (2) genome-wide imputation based on genotypic data from both WES (for better accuracy) and SNP array (for better coverage) is better than that based on WES data alone. After the aggregation process (Supplementary Note), \(\sim 1,203,000\) variants were retained in the merged VCF file.

Genotype imputation. We used the Michigan Imputation Server (Minimap3)\(^3\) for genotype imputation \((n = 1,740)\). The HaploTypo Reference Consortium (HRC, r1.1 2016)\(^{4}\) was used as the reference panel, Eagle v.2.3 for phasing and the European population for quality control. After the postimputation process (Supplementary Note and Supplementary Fig. 10), we obtained \(\sim 14,079,000\) polymorphic variants in our cohort. We evaluated the suitability of the HRC reference panel for cross-ethnicity genotype imputation in our study using 196 Ashkenazi Jewish individuals in our cohort for whom the whole-genome sequencing (WGS) data were available. The genotype imputation we performed was highly accurate: in 183 individuals (out of 196), genotypes of \(>99\%\) of \(2,020\) randomly selected noncoding variants that were not genotyped by either WES or SNP array data were correctly imputed (Supplementary Fig. 11).

Polygenic risk score analysis. We calculated PRS using PRSice-2 (refs. 2,6,7) to analyze disease risk from common variants in our longevity cohort. We first collected summary statistics from the most recent GWAS of seven complex diseases of European or predominantly European ancestry: AD\(^1\), CAD\(^2\), T2D\(^2\), stroke\(^2\), prostate cancer\(^2\), breast cancer\(^2\) and pancreatic cancer\(^2\). From combined genotype data after imputation for 1,740 samples, common SNPs (MAF > 5%) were imputed in the cohort and linkage disequilibrium (LD) clumping was carried out if they were within 250 kb and \(R^2 > 0.1\). After clumping, we used 19 P-values (1.00, 0.90, 0.80, 0.70, 0.60, 0.50, 0.40, 0.30, 0.20, 0.10, 0.01, 1 \times 10^{-6}, 1 \times 10^{-8}, 1 \times 10^{-10}, 1 \times 10^{-12}, 1 \times 10^{-14}\) and \(1 \times 10^{-16}\) as cutoffs to select SNPs for scoring and, for AD, additional ones to restrict selection to most AD-associated SNPs. After removal of outliers based on MDS analysis (Supplementary Fig. 12), non-Ashkenazi Jewish individuals and kinship, 910 centenarians and controls among 1,740 samples were used to evaluate the association between disease PRS and longevity in the cohort (Extended Data Fig. 10a). To remove population substructure and sex difference, we included the top ten MDS components derived from common SNPs in the combined phenotype dataset and gender as covariates in the regression analysis to evaluate PRS association. When analyzing PRS of prostate cancer and breast cancer, only male and female individuals were considered, respectively.

Rare variant association analysis. Among 2,021 Ashkenazi Jewish individuals with WES data, 536 were centenarians and 506 were controls. Pairs of individuals with the proportion of alleles shared identity-by-descent (IBD) \(>0.4\) were identified as related—that is, non-zygotic twins, parents and children and full siblings—and one sample per pair was excluded, with inclusion to achieve more cases, higher ages of cases and lower ages of controls. In our study cohort we identified 31 participants as being related to other participants due to high IBD. After excluding these, we had 515 cases (mean age 101 years) and 496 controls (mean age 83 years) for rare variant association analysis (Table 1 and Extended Data Fig. 10b). In this study, we analyzed rare variants with alternative allele frequencies <1% in Ashkenazi Jewish populations, which were calculated based on the average allele frequency in 731 unrelated (to first-degree kinship) Ashkenazi Jewish individuals in our centenarian cohort (2,021) (excluding 2,021 other individuals included in our study (Table 1)) and that in Ashkenazi Jews reported in gnomAD. Longevity association was assessed at the variant, gene and gene set levels. We evaluated the longevity association of each rare coding variant using the Firth logistic regression\(^5\). For association tests at gene and gene set levels, we performed the burden test and SKAT (implemented in R v.1.3 (ref. 66) to test the longevity association of six different subsets of rare variants within each gene or gene set. The variant-masking scheme\(^6\) was designed to group similar rare variants of specific properties based on CADD (v.1.4) and PrimaiAI (v.0.2) annotation. CADD is widely used as a variant annotation tool to predict the functionality (that is, being functional or neutral) of variants. In contrast, PrimaiAI predicts their clinical impact (that is, being pathogenic or benign). We defined different classes of variant based on the recommended thresholds of CADD and PrimaiAI scores (Supplementary Table 4): all rare variants (without masking), functional (or non-neutral)\(^7\) rare variants (CADD \(>20\) dominant, pathogenic rare variants (PrimaiAI score \(>0.8\)), recessive pathogenic rare variants (PrimaiAI score \(>0.7\)), functional but dominant benign rare variants (CADD \(>20\) and PrimaiAI score \(>0.8\)) or recessive benign rare variants (CADD \(>20\) and PrimaiAI score \(<0.5\)). The minimum \(P\) -value\(^8\) was used to combine \(P\) values of the aforementioned six sets of rare variants at the gene or gene set level. For gene-based association tests, only genes with multiple rare variants after masking were tested for the corresponding rare variant category. In total, 15,935 genes were tested for each one variant category. For gene set association, we compiled 20 gene sets of aging pathways for nine aging hallmarks\(^1\) (Supplementary Table 6) and used the burden test and SKAT to test the longevity association of those six sets of rare variants within each of those 20 gene sets. FDR was used to correct for 130,297 P-values at the gene level, 31,870 (2 \times 15,935) combined P-values at the gene level and 40 (2 \times 20) combined P-values at the gene set level. For rare variant association at the gene set level, we conducted an independent test using the same framework but in two subcohorts, APOE4\(^+\) and APOE4\(^−\). FDR was used to correct for 80 (2 \times 20) combined P-values in this analysis. Gender and top ten MDSs were included as covariates in all rare variant association analyses in the discovery cohort.

Network/pathway enrichment of rare variants. In addition to conventional approaches to the study of rare variant association, we investigated whether longevity-associated rare variants aggregate in a gene network and pathways. We first used IGS\(^+\) to score longevity-associated genes by integration of rare-variant association tests at the gene level with gene functional network\(^6\). To consider information of all rare coding variants in IGS\(^+\) scoring, we collected gene association signals by applying the weighted burden test (using the R package SKAT)\(^6\) on rare coding variants of each gene weighted by the corresponding CADD scores. We then tested whether the top 100 genes tend to be scored higher than the top 100 genes derived from randomized rare variant association signals using the Wilcoxon rank-sum test. We combine the top 100 genes implicated by longevity association of rare variants and the functional gene network in an unbiased manner, we first performed pathway enrichment analysis using TopGene Suite\(^8\), in which 1,245 pathways from different pathway databases were analyzed concurrently to summarize the top enriched pathways.
across pathway databases. In addition, we compared the top enriched KEGG and Reactome pathways identified by ToppGene Suite and three other widely used tools for pathway enrichment analysis—Enrichr, gProfiler and GSEA—to derive enriched pathways supported by multiple analysis tools.

**Lifespan analysis of rare variants.** In our longevity cohort, after removal of kinship relatedness we had the date of death—and thus definitive lifespan information—for 553 Ashkenazi Jewish individuals (202 males and 351 females) (Table 1 and Extended Data Figs. 8 and 10), among which were 550 (~99.5%) individuals with a lifespan ≥65 years. Since no censored data were included in our lifespan cohort—that is, all subjects reached the endpoint (death)—for all lifespan analyses of rare variants we tested the association between lifespan and the burden of rare variants in the lifespan cohort using a unified accelerated linear model \( \log(t) \) with the log-transformed age at death as the outcome and gender as a covariate. Different from rare variant association analyses that aim to discover longevity-associated rare variants using a longevity case-control design, our lifespan analyses of rare variants investigated how pathogenic and protective rare variants discovered in our case-control study impact human lifespan through quantitative analyses.

**Pathogenic rare variants and lifespan.** We investigated whether pathogenic rare variants can adversely affect lifespan. We used PrimateAI\(^{12}\), which was specially designed and optimized for prediction of disease-causing variants\(^{13}\), to select highly pathogenic rare coding variants using a stringent score threshold of \( \geq 0.9 \), and assessed how the total count of their alternative alleles (the exome-wide burden) affected lifespan. PrimateAI is a learning algorithm that expands the dataset for training by inclusion of common variants from nonhuman primates to improve the power for prediction of human pathogenic variants. No direct comparison of variant effects on longevity was made between human and nonhuman primates using PrimateAI.

**Protective rare variants and lifespan.** Our rare-variant association tests uncovered a burden of rare variants in Wnt signaling genes that may have pro-longevity effects among APOE\(^{+}\) (Supplementary Table 14). We investigated their impact on lifespan by examining those protective rare variants in our lifespan cohort through several analyses. We evaluated whether the alternative allele count of those protective rare variants in Wnt signaling genes is correlated with lifespan among APOE\(^{+}\) and APOE\(^{-}\); from a complementary angle, we investigated whether APOE differentially affects the lifespan of individuals with a high or low burden of those protective rare variants; and, finally, we also examined centenarians and noncentenarians separately in the lifespan analysis to differentiate it from the association study in which longevity status was used.

**Replication studies.** To maximize the extent of replicating human longevity association of rare variants, we prepared longevity case-control replication studies using cohort-specific criteria of determination of longevity cases and controls. First, longevity cases are individuals older than human life expectancy. Second, longevity controls are individuals substantially (>15 years) younger than cases. We used the WES data from three cohorts—a German longevity cohort, a UK Biobank longevity cohort and a longevity cohort from ADSP—to replicate the longevity association of rare variants discovered in our Ashkenazi Jewish longevity cohort. The German sample comprised 1,265 long-lived individuals (age range 94–110 years, mean age 99 years) as described previously\(^{14}\), and 4,195 younger controls (mean age 35 years) recruited as part of the FoCus discovery study in the replication analysis. We tested the six masking groups of APOE\(^{+}\) individuals for pathway enrichment analysis—Enrichr, gProfiler and GSEA—to derive enriched pathways supported by multiple analysis tools.

**Ultra-rare variants and lifespan.** To maximize the extent of replicating human longevity association of rare variants, we prepared longevity case-control replication studies using cohort-specific criteria of determination of longevity cases and controls. First, longevity cases are individuals older than human life expectancy. Second, longevity controls are individuals substantially (>15 years) younger than cases. We used the WES data from three cohorts—a German longevity cohort, a UK Biobank longevity cohort and a longevity cohort from ADSP—to replicate the longevity association of rare variants discovered in our Ashkenazi Jewish longevity cohort. The German sample comprised 1,265 long-lived individuals (age range 94–110 years, mean age 99 years) as described previously\(^{14}\), and 4,195 younger controls (mean age 35 years) recruited as part of the FoCus discovery study in the replication analysis. We tested the six masking groups of APOE\(^{+}\) individuals for pathway enrichment analysis—Enrichr, gProfiler and GSEA—to derive enriched pathways supported by multiple analysis tools.

**Ultra-rare variants and lifespan.** To maximize the extent of replicating human longevity association of rare variants, we prepared longevity case-control replication studies using cohort-specific criteria of determination of longevity cases and controls. First, longevity cases are individuals older than human life expectancy. Second, longevity controls are individuals substantially (>15 years) younger than cases. We used the WES data from three cohorts—a German longevity cohort, a UK Biobank longevity cohort and a longevity cohort from ADSP—to replicate the longevity association of rare variants discovered in our Ashkenazi Jewish longevity cohort. The German sample comprised 1,265 long-lived individuals (age range 94–110 years, mean age 99 years) as described previously\(^{14}\), and 4,195 younger controls (mean age 35 years) recruited as part of the FoCus discovery study in the replication analysis. We tested the six masking groups of APOE\(^{+}\) individuals for pathway enrichment analysis—Enrichr, gProfiler and GSEA—to derive enriched pathways supported by multiple analysis tools.

**Data availability**

All summary statistics for the longevity association of rare coding variants in our Ashkenazi Jewish longevity cohort are available at http://zdzlab.einsteinmed.org/1/longevity.html. Due to privacy concerns for our research participants, individual-level genetic data from the Einstein longevity study are not publicly available; however, anonymized data will be shared by request from a qualified academic investigator, providing the data transfer is approved by the Institutional Review Board and regulated by a material transfer agreement. The German longevity cohort data are part of the PopGen Biobank (Schleswig-Holstein, Germany) and can be accessed through a Material Data Access Form (http://www.dkab.de/p2n/Information+for+Researchers.html). Sequence and phenotype data of the UK Biobank and ADSP cohorts are available at https://bbams.ndph.ox.ac.uk/ams and https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000572, respectively. All software used in our analyses was open source and is described in Methods.

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Author contributions
J.-R.L. and Z.D.Z. conceived the formal analysis. J.-R.L. executed the formal analysis. P.S.-C. and A.S. obtained the study resources. Z.D.Z., P.S.-C., J.M., Q.Z. and T.G. performed data curation. Z.W. performed variant imputation. V.N., G.G.T., M.D.G., A.F., A.N. and S.G. participated in replication analysis. Z.D.Z. and N.B. conceived of the research goals and acquired funding. J.-R.L. and Z.D.Z. wrote the original draft. J.V., Y.S., S.M., P.D.R., L.J.N., W.C.L., V.G., K.Y., G.A., M.L., M.R.J. and N.N. participated in review and editing. The R.G.C. performed WES and SNP array genotyping.

Competing interests
J.V. is a founder of Singulomics Corp. P.D.R. and L.J.N. are cofounders of NRTK Biosciences. All other authors declare no competing interests.

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Extended Data Fig. 1 | The replication study of gene-set longevity association using the WES data of the German longevity cohort. The longevity case-control study consists of 1,265 longevity cases and 4,195 longevity controls. $P^*$ denotes $P$-value corrected for 12 categories of rare variants using the minimal-$P$ value test from Flannick et al. (Methods). The text for the significant association denotes the lowest raw $P$-value among different groups of tested rare variants and FDR. (A) Full longevity cohort. (B) APOE4 stratified cohorts.
Extended Data Fig. 2 | The replication study of gene-set longevity association using the UK Biobank WES data. The longevity case-control study consists of 104 cases with at least one parent age at death ≥ 100 years and 23,405 controls with both parent age at death < 95 years. P* denotes P-value corrected for 12 categories of rare variants using the minimal-P value test from Flannick et al.67 (Methods). The text for the significant association denotes the lowest raw P-value among different groups of tested rare variants and FDR. (a) Full longevity cohort. (b) APOE4 stratified cohorts.
Extended Data Fig. 3 | The replication study of gene-set longevity association using the ADSP WES data. The longevity case-control study consists of 1,121 non-AD cases with age ≥ 90 years and 38 non-AD controls with age < 75 years. $P^*$ denotes $P$-value corrected for 12 categories of rare variants using the minimal-$P$ value test from Flannick et al. (Methods). The text for the significant association denotes the lowest raw $P$-value among different groups of tested rare variants and FDR.
Extended Data Fig. 4 | Gene-set rare variant association in the APOE4-stratied cohorts of the discovery (Ashkenazi Jewish) longevity cohort. 

$P^*$ denotes $P$-value corrected for 6 categories of tested variants using the minimal-$P$ value test from Flannick et al.67 (Methods). The text for the significant association denotes the lowest raw $P$-value among different groups of tested rare variants and FDR.
**Extended Data Fig. 5 | Lifespan analysis of protective variants in WNT signaling genes for noncentenarians.** *P* denotes uncorrected *P*-value derived from linear regression with the log-transformed age at death as the outcome and the gender as a covariate (See Methods). ‘WNT low’ and ‘WNT high’ represent the alternative allele count of rare variants in WNT signaling genes ≤ 1 and > 1 (the median), respectively. In parentheses are the numbers of individuals. MD stands for ‘median difference’. The asterisk denotes FDR < 0.05. (a) The lifespan difference of individuals carrying a high and low burden of protective rare variants in WNT signaling genes. (b) Negative effects of APOE4 on lifespan with high and low burden of protective rare variants in WNT signaling for noncentenarians.
Extended Data Fig. 6 | Lifespan analysis of protective variants in WNT signaling genes for centenarians. P denotes uncorrected P-value derived from linear regression with the log-transformed age at death as the outcome and the gender as a covariate (See Methods). ‘WNT low’ and ‘WNT high’ represent the alternative allele count of rare variants in WNT signaling genes ≤ 1 and > 1 (the median), respectively. In parentheses are the numbers of individuals. MD stands for ‘median difference’. (a) The lifespan difference of individuals carrying a high and low burden of protective rare variants in WNT signaling genes. (b) Negative effects of APOE4 on lifespan with high and low burden of protective rare variants in WNT signaling for centenarians.
Extended Data Fig. 7 | Disease-PRS analyses for centenarian and control. This shows the results of PRS analyses for age-related diseases in the centenarian cohort. In the boxplots, points represent individuals, and horizontal lines represent upper fence (maximum in Q3 + 1.5xIQR), upper quartile (Q3), median, lower quartile (Q1), lower fence (minimum in Q1 - 1.5xIQR), sequentially from top to bottom; IQR: interquartile range (25th to the 75th percentile). n = 910 biologically independent samples in the boxplots on the right panels for coronary artery disease, type 2 diabetes, stroke, and pancreatic cancer. n = 339 and 571 biologically independent samples in the boxplots on the right panels for prostate cancer and breast cancer, respectively. Above the boxplot on the right are raw and adjusted (in parentheses) P-values for the best prediction in the Nagelkerke’s R² plot on the left, which were calculated based on logistic regression and the permutation test in PRSice-2, respectively. For stroke, breast cancer, prostate cancer, and pancreatic cancer, no robust association was observed between their PRS and the longevity status as originally defined in our cohort. (a) Coronary artery disease. (b) Coronary artery disease without considering SNPs within 1Mbps of rs7412 or rs429358 (SNPs for the APOE haplotype). (c) Type 2 diabetes. (d) Stroke. (e) Prostate cancer. Only males are considered. (f) Breast cancer. Only females are considered. (g) Pancreatic cancer.
Extended Data Fig. 8 | Basic statistics of the lifespan cohort. (a) Lifespan distribution of 553 individuals. (b) Survival curves of 202 males and 351 females composing the analyzed cohort. Females have a significant survival rate than males based on cox regression model ($P=1.71E-07$; coxph in R package).
Extended Data Fig. 9 | Correlation between lifespan and common-variant genetic risk of age-related diseases. *P*-values were based on the result of linear regression (regress log lifespan on genetic disease risk) corrected for gender. (a) Alzheimer’s disease. The plots on the left and right show the boxplot and survival curves of APOE4+ and APOE4−, respectively. MD stands for ‘Median Difference’. In the boxplots, points represent individuals, and horizontal lines represent upper fence (maximum in Q3+1.5×IQR), upper quartile (Q3), median, lower quartile (Q1), lower fence (minimum in Q1−1.5×IQR), sequentially from top to bottom; IQR: interquartile range (25th to the 75th percentile). n = 553 biologically independent samples. (b) Coronary artery disease. *r* represents ‘correlation coefficient’. (c) Type 2 diabetes.
Extended Data Fig. 10 | Flowcharts of sample collection for different analyses.  

**a** Flowchart of sample collection for PRS analyses and lifespan analyses of rare variants and disease PRS. Refer ‘Rare variant association analysis’ subsection for the strategy of removing kinship for PRS analysis that involves longevity status. The strategy of removing kinship in lifespan analyses is to randomly exclude one in pairs of individuals with the proportion of alleles shared identity-by-descent (IBD) > 0.4.  

**b** Flowchart of sample collection for rare variant association tests, network-integrated analyses, and lifespan analyses of rare variants (and APOE4).
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Software and code

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Data collection

No specific software was used to collect the data.

Data analysis

All used softwares in our analyses were open source and were described in the Methods section of the manuscript.

Plink 1.9, PRSice-2 2.1.1, SKAT (R package 1.3), ADDIE 1.4, PrimateAI 0.2, Michigan Imputation Server (https://mpower.genetics.med.umich.edu/index.html), iGSP (https://zenodo.org/record/1034362#.X-jwQNgzY2w), ToppGene Suite (https://toppgene.uchc.edu/), Enrichr (https://maayanlab.cloud/Enrichr/), g:Profiler (https://biit.cs.ut.ee/gprofiler/gost), GSEA (http://www.gsea-msigdb.org/gsea/msigdb/annotate.jsp).

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All summary statistics for the longevity association of rare coding variants in our Ashkenazi Jewish longevity cohort are available at http://zdzlab.einsteinmed.org/1/longevity.html. Due to privacy concerns for our research participants, individual-level genetic data from the Einstein longevity study are not publicly available; however, anonymized data will be shared by request from a qualified academic investigator as long as the data transfer is approved by the Institutional Review Board and regulated by a material transfer agreement. The German longevity cohort data are part of the PopGen Biobank (Schleswig-Holstein, Germany) and can be accessed through a Material Data Access Form (http://www.uksh.de/p2n/Information+for+Researchers.html). Sequence and phenotype data of the UK Biobank and
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf.

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- Sample size: No statistical methods were used to predetermine sample size as all the available samples from the WES data were considered.
- Data exclusions: No data were excluded with pre-established criteria in favor of results.
- Replication: We used three independent longevity cohorts in which we successfully replicated our finding on longevity association of rare variant in aging pathways.
- Randomization: The experiments were not randomized as this approach was not relevant to the study design.
- Blinding: The investigators were not blinded to allocation during experiments and outcome assessment as this was not relevant to the study design.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | Involved in the study |
| ☒ Antibodies | ☒ ChIP-seq |
| ☒ Eukaryotic cell lines | ☒ Flow cytometry |
| ☒ Palaeontology and archaeology | ☒ MRI-based neuroimaging |
| ☒ Animals and other organisms | ☒ Human research participants |
| ☒ Clinical data | ☒ n/a |
| ☒ Dual use research of concern | ☒ n/a |

Human research participants

Policy information about studies involving human research participants.

Population characteristics

The discovery cohort is Einstein Longevity cohort: Longevity cases were defined as individuals age ≥ 95 years, and individuals age < 95 without a parental history of longevity (neither parent survived beyond 95 years of age) as controls.

To maximize the extent of replicating human longevity association of rare variants, we prepared three longevity case-control replication studies using cohort-specific criteria of determining longevity cases and controls. German longevity cohort: Longevity cases are long-lived individuals (age: 94-101 years), and controls are blood donors [mean age: 35 years]; UK Biobank longevity cohort: Longevity cases are individuals with at least one long-lived parent (lifespan ≥ 100 years), and controls are individuals with parents of usual survival (lifespan < 95 years); ADSP longevity cohort: Longevity cases are individuals with age ≥ 90 years, and control are individuals with age < 75 years.

In all longevity case-control studies, gender and top 10 principal components from PCA or MDS derived from common variants were considered as population characteristics and included as covariates in our analyses.

Recruitment

The discovery study subjects were Ashkenazi Jewish participants from two longevity cohorts, the Longevity Genes Project (LGP) and the LonGenity study, who were recruited and characterized at the Albert Einstein College of Medicine since 1998. The German longevity subjects consist of long lived individuals previously described [PMID: 27004735] and blood donors at the University Hospital Schleswig Holstein in Kiel and Lübeck, Germany. The UK Biobank longevity subjects were recruited from general population as previously described [PMID: 33087929]. The ADSP longevity subjects were recruited from a study...
cohort for Alzheimer’s disease.

| Ethics oversight |
|------------------|
| The Einstein WES data: The Institutional Review Board at Albert Einstein College of Medicine |
| The German WES data: The Ethics Committee at the Medical Faculty of Kiel University |
| The UKB WES data: The Ethics Advisory Committee and the external ethics committees |
| The ADSP WES data: Broad Institute, the Baylor College of Medicine’s Human Genome Sequencing Center, and Washington University’s McDonnell Genome Institute |

Note that full information on the approval of the study protocol must also be provided in the manuscript.