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Original Article

# Exome-Wide Association Study Identifies *FN3KRP* and *PGP* as New Candidate Longevity Genes

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## Abstract

Despite enormous research efforts, the genetic component of longevity has remained largely elusive. The investigation of common variants, mainly located in intronic or regulatory regions, has yielded only little new information on the heritability of the phenotype. Here, we performed a chip-based exome-wide association study investigating 62 488 common and rare coding variants in 1248 German long-lived individuals, including 599 centenarians and 6941 younger controls (age < 60 years). In a single-variant analysis, we observed an exome-wide significant association between *rs1046896* in the gene fructosamine-3-kinase-related-protein (*FN3KRP*) and longevity. Noteworthy, we found the longevity allele C of *rs1046896* to be associated with an increased *FN3KRP* expression in whole blood; a database look-up confirmed this effect for various other human tissues. A gene-based analysis, in which potential cumulative effects of common and rare variants were considered, yielded the gene phosphoglycolate phosphatase (*PGP*) as another potential longevity gene, though no single variant in *PGP* reached the discovery *p*-value (1 × 10E–04). Furthermore, we validated the previously reported longevity locus cyclin-dependent kinase inhibitor 2B antisense RNA 1 (*CDKN2B-AS1*). Replication of our results in a French longevity cohort was only successful for *rs1063192* in *CDKN2B-*

*AS1*. In conclusion, we identified 2 new potential candidate longevity genes, *FN3KRP* and *PGP* which may influence the phenotype through their role in metabolic processes, that is, the reverse glycation of proteins (*FN3KRP*) and the control of glycerol-3-phosphate levels (*PGP*).

**Keywords:** Association study, Healthy aging, HumanExome BeadChip, Long-lived individuals, Rare variants

Human longevity is a complex phenotype influenced by both genetic and environmental factors, which furthermore interact, for example, via epigenetic changes (1). Heritability estimates for longevity range from 12% (2) to 30% (3). However, in the oldest-old, the genetic influence appears to be even higher and it has been suggested to be as high as 48% (4). Candidate studies have yielded many potential longevity associations, but most of them have not been replicated in independent investigations. To date, variation in only 4 loci has been confirmed to influence longevity across populations: *APOE* (5,6), *FOXO3* (7,8), the 5q33.3 locus (9,10), and *CDKN2B-AS1* (11–13). The first 3 reached genome-wide significance in large genome-wide association studies (GWAS) (9,14). However, the identified variants together explain only a small proportion of the longevity heritability. Thus, novel approaches are needed to identify additional loci involved in the phenotype.

Longevity studies have mostly been conducted following the common disease/common variant hypothesis, which is based on the assumption that the probability of becoming long-lived depends on a small number of single-nucleotide variants (SNVs) that occur at high frequency in all populations. Yet, it has been estimated that common variants explain only 5%-10% of the heritability of complex traits (15). Therefore, rare variants (minor allele frequency [MAF] < 0.01) may play an important role in the genetic regulation of longevity, but they are often not covered by genome-wide genotyping arrays or imputations and are therefore less studied (14). Common variants often reside in regulatory/intronic regions (16), whereas rare variants are more likely to be present in exons. Therefore, an effective way to target such rare variants is through exome-based genotyping or through whole genome or exome sequencing. Some studies have used these methods to investigate coding region variants associated with longevity (eg, (17–20)). These approaches yielded important results, not only in terms of new longevity variants in, for instance, *CLEC3B* and *HLA-DQB1* (17,19), but also provided novel analysis methods that could extend the discovery spectrum of loci and genes influencing complex traits, like the combined analysis of multiple variants (11).

Here, we performed an exome-wide association study in a large German longevity cohort that comprised more than 1200 long-lived individuals (LLI) and 6941 younger controls using the Illumina Infinium HumanExome BeadChip that covers both rare and common variants to identify novel variants/genes associated with longevity.

## Method

### Study Populations

In the following, a brief overview of the populations used in this study is presented. For a more extensive description, please refer to the Supplementary Material. Our discovery cohort comprised 1248 German LLI (male/female ratio: approximately 1/3; age range: 94–110 years; mean age: 99 years) and 6941 younger controls (age < 60 years). For replication, we investigated a Danish data set with 1002 cases (male/female ratio: 1/3; age range: 90.0–102.5 years; mean age: 97.4 years) and 738 controls (mean age: 66.3 years), and a French cohort with 1264 LLI (male/female ratio ~ 1/4.5, age range 91–115+ years; mean age 102.4 years;) and 1830 younger subjects (age < 62 years). All individuals belonged to the top 1% of the survivors of their respective birth cohorts.

### Exome Chip Genotype Calling and Quality Control in the German Study Population

The samples were genotyped on the Infinium HumanExome-12v BeadChip (244,770 SNVs) (Illumina Inc., San Diego, CA; Supplementary Figure 1). Quality control was done with the software Plink 1.9 (21). In total, 224 samples were removed because they had failed one or more of the following inclusion criteria: concordant sex information, missing genotype < 3%, heterozygosity rate greater or lower than ± 4*SD* from the mean, and no relatedness of individuals. Relatedness was estimated using the identity by descent (IBD) metric (22). In case of relatives (IBD > 0.1875; halfway between the expected IBD for third- and second-degree relative (22)), only one individual was included in the analysis. SNVs were excluded if the missing rate was too high (> 3%) or, for common SNVs in the control sample, if they deviated from the Hardy–Weinberg equilibrium (HWE) ($p < .0001$). Due to the lack of sufficient statistical power, SNVs with an extremely low minor allele frequency (MAF < 0.003) were removed prior to the single-variant analysis. This resulted in 62 488 remaining variants.

Population substructure was evaluated with the principal component analysis (PCA) using a common set of independent markers (HapMap3 ancestry set from 4 ethnic populations; 16 782 SNVs). The principal components (PC) were calculated using PLINK 1.9 (21). The first 5 PCs were selected for further association analysis to adjust for population stratification. Additionally, stratification outliers were identified based on the local outlier factor (LOF > 1.7) (23) and excluded to mitigate population stratification.

### Association Analysis in the German Sample

Single-variant association analysis was performed using the logistic regression test implemented in PLINK 1.9 (21) assuming an additive genetic model and additional influence variables, namely the 5 PCs and sex (Supplementary Figure 1). Candidate longevity SNVs with a discovery *p*-value < 1 × 10E–04 were selected for replication. Relaxation of the significance threshold allows the identification of longevity SNVs that usually have small effects on the phenotype (24). To identify additional association signals and to test for independency of the newly identified SNVs from the effects of the known longevity-associated locus *TOMM40/APOE/APOC1* (9,11,25), a conditional association test was performed using logistic regression in PLINK 1.9 (21), adjusting for the SNVs *rs2075650*, *rs4420638*, and *rs769449*. The Sanger imputation service (http://www.sanger.ac.uk/science/tools/sanger-imputation-service) and the 1000 Genomes phase I v.3 reference panel were used to enrich the pool of common SNVs.

In addition to the single-variant association testing, a gene-based analysis was performed. All SNVs that passed quality control (112,977 SNVs) were used to generate the gene set (Supplementary Figure 1). Potential cumulative effects of rare and common variants with longevity were tested using both burden and non-burden (ie,

SKAT) approaches (application of the RC-SKAT algorithm from the R-package SKAT (26)). Both approaches were considered because burden tests were shown to perform better when multiple variants in a region are causal and influence the phenotype in the same direction (27), while non-burden tests, like SKAT (28), are more advantageous when SNVs in the region interact or show opposing directions of effect (29). The overall effect of rare and common variants in a gene was evaluated based on the adaptive sum test (26) in combination with either burden or SKAT. Concordantly, the gene-based Bonferroni-corrected $p$-value threshold was based on the number of gene sets tested: $p < 3.3 \times 10E{-}06$ (significance threshold of 0.05/14 790 genes = the number of genes with SNVs that remained after quality control).

Epistatic interaction between pairs of candidate SNVs was assessed using multifactor dimensionality reduction (MDR) analysis (30) correcting for confounders such as sex and the known longevity-associated locus *TOMM40/APOE/APOC1* (9,11,25). The entropy-based clustering algorithm used by MDR calculates case–control ratios for each of the possible multilocus genotypes; therefore, if a genotype combination is more abundant in cases than in controls, it is considered as a high-level interaction. The MDR interaction model describes the percentage of entropy (information gain) by each SNV or SNV interaction. Positive values of entropy indicate synergistic or nonadditive interactions, while negative entropy values indicate redundancy between the markers or lack of synergy between the interacting markers. The model (ie, combination of SNVs) that appeared most consistently among replicates in the training balance accuracy level was considered the best model. Significance was calculated via permutation tests using 1000 permutations and a significance level of $\alpha = 0.05$. MDR was implemented employing the open-source MDR software package version 3.0.2 (https://www.mybiosoftware.com/mdr-2-0-multifactor-dimensionality-reduction.html) (31).

The functional implications of the longevity variants were assessed using our previously published gene expression data from whole blood samples of 55 LLI and 73 control individuals (independent cohort from Germany) (32) as well as publicly available data from the Blood eQTL browser (https://genenetwork.nl/bloodeqtlbrowser/) (33) and the GTEx eQTL (https://www.gtexportal.org/home/; accessed April 5, 2019).

### Genotyping in the Replication Cohorts

The Danish LLI were genotyped using the Illumina HumanOmniExpress Array (Illumina Inc.). Preimputation quality control included filtering of SNVs on genotype call rate <95%, HWE $p < 10E{-}04$, MAF < 1% and filtering of individuals on sample call rate <95%, relatedness, and sex mismatch. After imputation to the 1000 Genomes phase I v.3 reference panel, genotype probabilities were converted to hard-called genotypes in PLINK 1.9 (using a cutoff of 90%) (21). For the Danish controls, data for the SNVs *rs1063192*, *rs1046896*, and *rs13119846* were extracted from quality-controlled genotype data (as above) created using the Illumina Infinium PsychArray (Illumina Inc.).

French individuals were genotyped by TaqMan (Thermo Fisher Inc., Waltham, MA) on a 7900HT Fast Real-time PCR System (Thermo Fisher Scientific Inc., Waltham, MA). Association analysis for the Danish and French cohort was performed with logistic regression accounting for sex as covariate using PLINK 1.9 (21).

### Availability of Data and Materials

All German samples and information on their corresponding phenotypes were obtained from the PopGen Biobank (Schleswig-Holstein, Germany). The data can be accessed through a Material Data Access Form (http://www.uksh.de/p2n/Information+for+Researchers.html).

## Results

### Single-Variant Association Analysis Reveals an Association of *rs1046896* in *FN3KRP* With Longevity

To identify new common and rare genetic variants associated with longevity with moderate to high effect size, a chip-based exome-wide association study was conducted using the Illumina Infinium HumanExome BeadChip. This array covers rare and common variants in a ratio of approximately 8:1 (34). In total, 1248 German LLI and 6941 younger controls were included in the study (Supplementary Figure 1). The single-variant analysis was performed based on 62 488 nonimputed SNVs, 1212 LLI, and 6762 younger controls who remained after quality control (Supplementary Figures 2 and 3). The single-variant association approach for rare and common variants yielded 11 candidate SNVs ($p < 1 \times 10E{-}04$; Table 1). The best association signal and the only one reaching exome-wide significance (except for the *TOMM40/APOE/APOC1* region, see below) was obtained for *rs1046896* in the gene *FN3KRP* (MAF = 0.32, $p = 7.40 \times 10E{-}07$; Table 1; Figure 1; Supplementary Table 1). In addition, we observed a longevity association for *rs1063192* in the *CDKN2B-AS1* region ($p = 2.99 \times 10E{-}05$; Table 1; Figure 1). Apart from these SNVs, we identified *rs2075650*, *rs4420638*, and *rs769449* in the *TOMM40/APOE/APOC1* region that is well known to be negatively associated with longevity (5,9,11,25).

The effects of the candidate variants were investigated for independency of the *TOMM40/APOE/APOC1* locus by a conditional association test. The results of the conditional analysis confirmed the independency of the longevity association of *rs1046896* (*FN3KRP*) as well as of the variants *rs55882518* (*NOTCH3*), *rs1063192* (*CDKN2B-AS1* region), *rs1319846* (*TMEM131L*), and *rs1790706* (*DSC2*) (Table 1). Additionally, an association analysis with the centenarian subpopulation ($n = 599$ individuals ≥100 years) was performed. The association analysis yielded higher ORs for *rs1046896* (*FN3KRP*), *rs55882518* (*NOTCH3*), *rs1063192* (*CDKN2B-AS1* region), and *rs184214819* (*SPZ1*) compared with the analysis using the whole study population (Supplementary Table 2). This effect has been reported before (35) and is explained by the larger genetic influence with increasing age. However, the centenarian subset comprised substantially fewer individuals and apart from *rs1046896* (*FN3KRP*), the longevity associations did not reach exome-wide significance (Supplementary Table 2).

Since the top hit, *rs1046896*, was located in a 3'UTR and might therefore affect gene expression in an allele-dependent manner, we investigated whether this SNV or SNVs in high LD ($r^2 > 0.8$ based on the CEU subpopulation from the 1000 Genomes Project Phase 3; Supplementary Table 3) influence local or distant gene expression. In our previously published whole blood transcriptome data from 55 LLI and 73 control individuals (independent cohort from Germany) (32), we observed significant cis-eQTL associations of *rs1046896* (and high LD SNVs) with the expression of *FN3KRP*. *FN3KRP* gene expression was higher in the presence of the *rs1046896* longevity allele C (major allele) (Figure 2A). Using publicly available data from the Blood eQTL browser (https://genenetwork.nl/

**Table 1.** Association Statistics for the 11 Longevity-Associated SNVs Identified by the Single-Variant Association Approach in the Whole German Study Population

| | | | MAF[a] | | | Basic Association Test | | Conditional Analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OR[b] | | OR[b] | |
| SNV | Gene | Chr | LLI | C | MA | [95% CI][c] | *p*[d] | [95% CI][c] | *p*[d] |
| *rs769449* | *APOE* | 19 | 0.056 | 0.109 | A | 0.48 [0.40–0.58] | 7.77E−15 | - | - |
| *rs4420638* | *APOC1* | 19 | 0.109 | 0.169 | G | 0.60 [0.52–0.69] | 3.55E−13 | - | - |
| *rs2075650* | *TOMM40* | 19 | 0.109 | 0.147 | G | 0.70 [0.61–0.80] | 3.51E−07 | - | - |
| *rs1046896* | *FN3KRP* | 17 | 0.276 | 0.324 | T | 0.78 [0.71–0.86] | 7.40E−07 | 0.77 [0.70–0.85] | 2.32E−07 |
| *rs55882518* | *NOTCH3* | 19 | 0.013 | 0.005 | T | 2.69 [1.73–4.18] | 1.07E−05 | 2.77 [1.78–4.30] | 6.23E−06 |
| *rs13119846* | *TMEM131L* | 4 | 0.486 | 0.438 | C | 1.22 [1.11–1.33] | 1.73E−05 | 1.21 [1.11–1.32] | 2.45E−05 |
| *rs1063192* | *CDKN2B-AS1* | 9 | 0.482 | 0.439 | G | 1.21 [1.11–1.32] | 2.99E−05 | 1.22 [1.12–1.33] | 1.08E−05 |
| *rs184214819* | *SPZ1* | 5 | 0.009 | 0.003 | A | 3.01 [1.77–5.11] | 4.72E−05 | 2.79 [1.64–4.74] | 1.52E−04 |
| *rs200956599* | *SKOR1* | 15 | 0.014 | 0.006 | T | 2.34 [1.55–3.54] | 5.24E−05 | 2.29 [1.52–3.47] | 8.65E−05 |
| *rs63750412* | *GRN* | 17 | 0.008 | 0.002 | T | 3.57 [1.93–6.61] | 5.18E−05 | 3.34 [1.80–6.18] | 1.29E−04 |
| *rs1790706* | *DSC2* | 18 | 0.159 | 0.189 | A | 0.79 [0.70–0.89] | 9.59E−05 | 0.78 [0.69–0.88] | 3.35E−05 |

*Note*: *APOC1* = apolipoprotein C1; *APOE* = apolipoprotein E; *CDKN2B-AS1* = cyclin-dependent kinase inhibitor 2B antisense RNA 1; *DSC2* = desmocollin 2; *FN3KRP* = fructosamine 3 kinase-related protein; *GRN* = granulin precursor; *NOTCH3* = notch 3; *SKOR1* = SKI family transcriptional corepressor 1; *SPZ1* = spermatogenic leucine zipper 1; *TMEM131L* = transmembrane 131 like; *TOMM40* = translocase of outer mitochondrial membrane 40.

C = controls; Chr = chromosome; LLI = long-lived individuals.

[a]Minor allele frequency, MAF; the definition of the minor allele (MA) is based on controls.

[b]Odds ratio for longevity, OR; based on the MA in controls.

[c]95% confidence interval, 95% CI; CI for the OR.

[d]Allelic *p*-values, calculated from logistic regression.

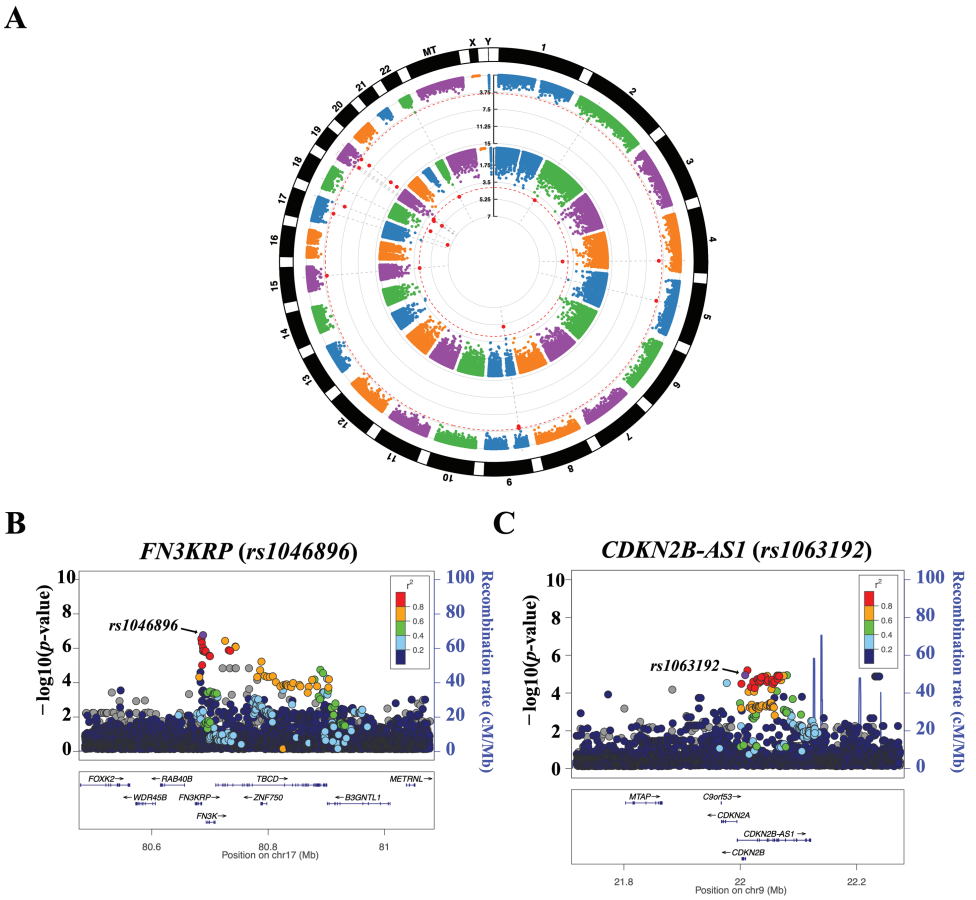


**Figure 1.** (**A**) Circular Manhattan plot summarizing the findings from the single-variant analysis. The inner plot represents the basic association results, the outer plot the association results after conditioning on the longevity-associated locus *TOMM40/APOE/APOC1*. The y-axis shows the -log(*p*-value), while the dotted line depicts the *p*-value threshold (1 × 10E−05). SNVs with $p < 1 \times 10E{-}05$ are shown as dots. (**B,C**). Regional plots for *FN3KRP* and *CDKN2B-AS1* candidate variants. Full color version is available within the online issue.

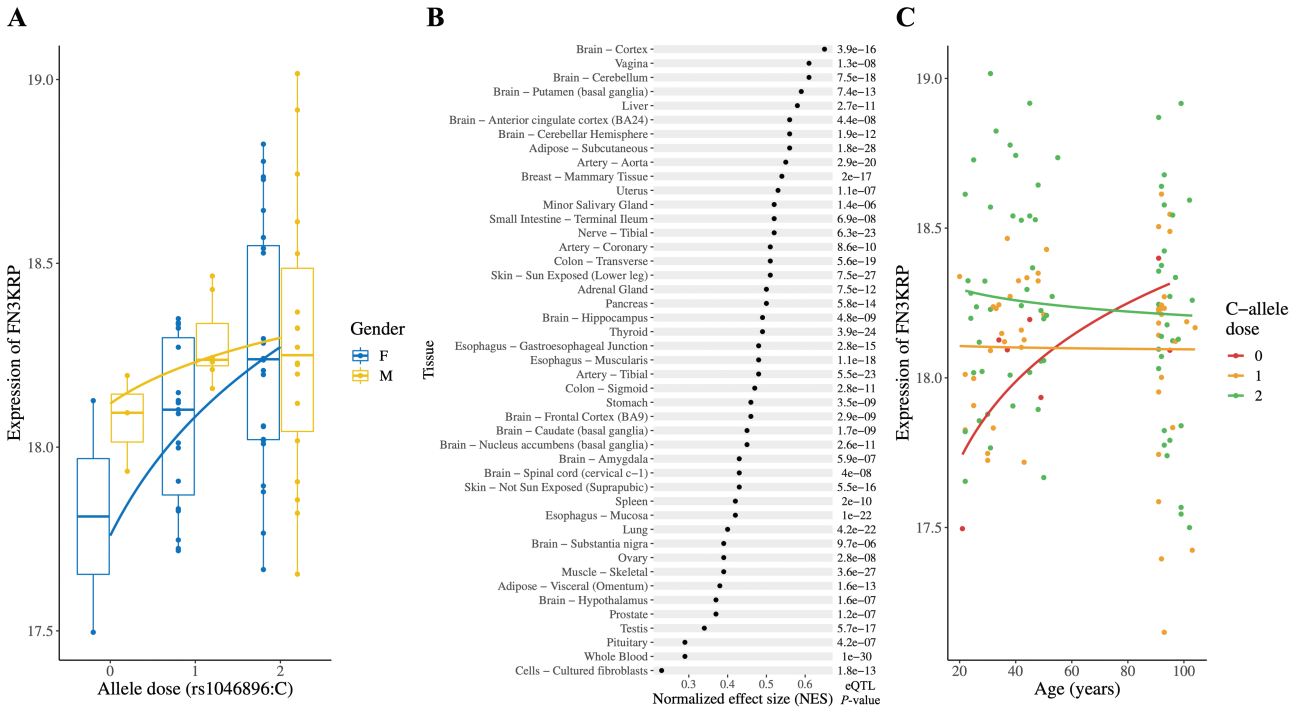


**Figure 2.** *rs1046896*-C allele-dose influence on *FN3KRP* mRNA expression. (**A**) Boxplots of *FN3KRP* expression according to C allele-dose shown separately for males (M) and females (F). (**B**) cis-eQTL effect of *rs1046896*-C on *FN3KRP* expression in several human tissues based on the GTEx Portal (GTEx Portal V8 Release, https://www.gtexportal.org). Normalized effect size (NES) is defined as the slope of the linear regression and is calculated as the effect of the allele *rs1046896*-C relative to *rs1046896*-T. eQTL *p*-value was calculated by adaptative permutation tests and adjusted using the false discovery rate. (**C**) Expression of *FN3KRP* for each individual as a function of age. Full color version is available within the online issue.

bloodeqtlbrowser/) (33) and the GTEx eQTL database (https://www.gtexportal.org/home/; accessed April 5, 2019), we confirmed the allele-dependent expression of *FN3KRP* in several tissues (eg, brain regions, testis, pancreas; Supplementary Table 4; Figure 2B). For the expression of *FN3KRP*, we observed a C-allele dose effect (TT<CT<CC) which was independent of age but more pronounced in females (Figure 2A and C). Additionally, LLI seemed to exhibit a high *FN3KRP* expression even when they were homozygous for the T allele (Figure 2C).

## The Longevity Association of *rs1063192* (*CDKN2B-AS1*) Replicates With Borderline Significance in an Independent Cohort

We aimed for replication of the association results in 2 independent cohorts. Sample sizes of the Danish and French cohorts (Danish: 1002 LLI, 738 younger controls; French: 1264 LLI, 1830 younger controls) limited the replication approach to the common variants *rs1046896* (*FN3KRP*), *rs1063192* (*CDKN2B-AS1*), and *rs1319846* (*TMEM131L*). The association of *rs1063192* (*CDKN2B-AS1*) reached borderline significance in the French ($p$ = .056 after Bonferroni correction (3 tests), odds ratio [OR] = 1.14; Table 2), but not in the Danish ($p$ = 1.00 after Bonferroni correction (3 tests), OR = 1.04; Table 2). The longevity associations of the other SNVs, including *rs1046896* in *FN3KRP*, did not replicate in the French or Danish (Table 2). Association tests performed with the centenarian subsets of all 3 cohorts yielded similar results (data not shown).

In a meta-analysis, we observed large inconsistency (I2) of the genetic effects across the 3 studies (I2 > 75%) for *rs1046896* (*FN3KRP*) and *rs1319846* (*TMEM131L*), but moderate I2 (25% < I2 <75%) for *rs1063192* (*CDKN2B-AS1*) (Table 2). The between-population heterogeneity of the genetic effects for *rs1063192* (*CDKN2B-AS1*) was estimated as 40.72% (Table 2). To account for potential heterogeneity biases, we used the random effects summary OR (OR(R)). The strongest evidence for an association with longevity was observed for *rs1063192* (*CDKN2B-AS1*) (OR(R) = 1.14, $p$ = .00174; Table 2).

The different ancestry of the individuals may have contributed to the heterogeneity of the results from the 3 cohorts. While the heterogeneity within the German sample was low and corrected for by PCA (see Methods section), we did not have equivalent genetic information on the French and Danish cohorts to correct for any influence of genetic heterogeneity.

## Gene-Based Analysis Reveals *PGP* as a Potential New Longevity Locus and Strengthens the *FN3KRP* Association

In addition to the single-variant association approach, we assessed the cumulative effects of common and rare variants within one genomic region. In the gene-based association test, 13 genes (apart from *TOMM40/APOE/APOC1*) were identified with an enriched burden of rare and common variants ($p$ < 1 × 10E–04). However, only *PGP* survived Bonferroni correction in both the SKATO and the burden test (Table 3). Of the 13 genes, 4 *(FN3KRP, GRN, SKOR1,* and *SPZ1*) had already been observed in the single-variant association analysis (Table 3). An association analysis using the centenarian subset only, yielded significant associations ($p$ < 3 × 10E–06) for 7 genes (apart from *APOE*) (Supplementary Table 5). Five genes (*TMEM14A*, *IRAK1BP1*, *ACPP*, *PLXNB1*, and *GAR1*) were

**Table 2.** Single-Variant Replication and Meta-analysis Statistics for Candidate SNVs in the French and Danish Populations

| SNV (Gene) | Sample | MAF[a] LLI | MAF[a] C | MA | Single-Variant Association Test OR[b] [95% CI][c] | $p$[d] | $pcorr$[d] | Meta-analysis $p(R)$[e] | OR(R)[f] | $Q$[g] | $I$[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *rs1046896* (*FN3KRP*) | German | 0.276 | 0.324 | T | 0.78 [0.71–0.86] | 7.40E–07 | | 5.54E–01 | 0.94 | 1.00E–04 | 89.35 |
| | French | 0.362 | 0.347 | | 1.06 [0.95–1.19] | 2.46E–01 | 7.38E–01 | | | | |
| | Danish | 0.278 | 0.288 | | 1.00 [0.85–1.17] | 9.94E–01 | 1.00 | | | | |
| *rs1063192* (*CDKN2B-AS1*) | German | 0.482 | 0.439 | G | 1.21 [1.11–1.32] | 2.99E–05 | | 1.74E–03 | 1.14 | 1.85E–01 | 40.72 |
| | French | 0.416 | 0.385 | | 1.14 [1.02–1.26] | 1.88E–02 | 5.62E–02 | | | | |
| | Danish | 0.485 | 0.476 | | 1.04 [0.90–1.20] | 6.36E–01 | 1.00 | | | | |
| *rs13119846* (*TMEM131L*) | German | 0.486 | 0.438 | C | 1.22 [1.11–1.33] | 1.73E–05 | | 4.10E–01 | 1.07 | 1.20E–03 | 85.22 |
| | French | 0.452 | 0.464 | | 0.93 [0.84–1.04] | 2.31E–01 | 6.92E–01 | | | | |
| | Danish | 0.456 | 0.444 | | 1.08 [0.94–1.24] | 3.05E–01 | 9.15E–01 | | | | |

*Notes*: Listed are rs-numbers, annotated gene name, chromosome, allele frequencies in cases and controls the minor allele, odds ratios with 95% confidence intervals and allelic $p$-values for each study population. The effective size of the German population was 1248 LLI and 6762 younger controls; for the French 1270 LLI and 1824 younger controls, and for the Danish 1002 LLI and 738 younger controls. *CDKN2B-AS1* = cyclin-dependent kinase inhibitor 2B antisense RNA 1; *FN3KRP* = fructosamine 3 kinase related protein; *TMEM131L* = transmembrane 131 like.

C = controls; LLI = long-lived individuals.

[a]Minor allele frequency, MAF; the definition of the minor allele (MA) is based on controls.

[b]Odds ratio for longevity, OR; based on the MA in controls.

[c]95% confidence interval, 95% CI; CI for the OR.

[d]Allelic $p$-values, calculated from logistic regression; Pcorr, corrected $P$-value using Bonferroni (corrected for 3 tests in the French and Danish study populations).

[e]Random-effects meta-analysis $p$-value; [f]Random-effects OR estimate; [g]$p$-value for Cochrane's Q statistic, Q; [h]I2 heterogeneity index (0–100).

identified in the centenarian subpopulation only, whereas *FN3KRP* and *HMHA1* overlapped with the results of the gene-based analysis using the entire German study population. Epistatic interactions among SNV candidates (Table 3) were assessed through MDR analysis. This analysis confirmed that *rs1046896* in *FN3KRP* had the largest univariate effect ($p < .01$) and that the CC genotype represented the favored allele combination for longevity. For the 2-locus interaction model, we observed a significant SNV-SNV interaction between *rs1046896-FN3KRP* and *rs2074442-HMHA1*, a gene associated with the immune response. For the 3-locus interaction model, we observed an interaction between the gene *rs2074442-HMHA1* and the 2 transcription regulators *rs2943549-HNF4G* and *rs4097-SET9* (Supplementary Figure 4).

## Discussion

In our German longevity cohort comprising more than 1200 LLI (incl. ~600 centenarians), we screened 62 488 common and rare exonic SNVs for an association with longevity. First, we found *rs1046896* in the 3'UTR region of *FN3KRP* to be associated with exome-wide significance. Second, we confirmed the *CDKN2B-AS1* region as a longevity locus and third, we identified *PGP* as a new candidate gene.

Here, we suggest *rs1046896* in *FN3KRP* as a novel longevity variant though the association only reached exome-wide significance in Germans. Single-association analysis (Figure 1B) revealed that *rs1046896* was surrounded by several other signals that were in high LD with *rs1046896* ($r^2 > 0.8$) and that displayed significant cis-eQTL associations (Supplementary Table 4). Despite the fact that *rs1046896* exhibited the strongest effect size, the gene-based analysis (Table 3, Supplementary Table 5) showed that the *FN3KRP* association was likely driven by more than just one SNV (5 SNVs in *FN3KRP* led to the significant association in the gene-based analysis). Additionally, the meta-analysis exposed a very high between-population heterogeneity for *rs1046896* (Table 2), suggesting that the SNV could be in different LD with the causal polymorphism in the 3 populations studied. Therefore, our findings indicate that *rs1046896* was not the only or main causative variant. This hypothesis could explain the heterogeneity with our replication results. Nevertheless, the longevity association with *FN3KRP* needs to be confirmed in larger or additional samples. An in-depth fine-mapping of the *FN3KRP* region would be the next necessary step to identify the true causative variant/s. Furthermore, we cannot exclude that other mechanisms (eg, epigenetics) may be involved in the regulation of *FN3KRP* expression. For instance, our observation suggests that a high *FN3KRP* expression seems to be linked to human longevity independent of *rs1046896*.

*FN3KRP* belongs to a gene family with an important role in the reversal of the nonenzymatic glycation of proteins (36). Glycation adversely affects protein functioning which leads, among other effects, to arterial stiffening (37) and to an accumulation of damaged proteins. This in turn promotes aging (36) and the development of age-related diseases (38). Both *FN3KRP* and fructosamine-3-kinase (*FN3K*), which shows 65% sequence similarity with *FN3KRP*,

**Table 3.** Association Statistics for the 16 Longevity-Associated Genes Identified by the Gene-Based Association Approach in the German Study Population

| | | | | SNVs | | | | |
|---|---|---|---|---|---|---|---|---|
| Gene | Chr | *P*_skato | *P*_burden | All | Tested | Rare | Common | NCBI rs identification number |
| *APOE* | 19 | 3.25E−15 | 3.25E−15 | 2 | 2 | 1 | 1 | *rs769449*, *rs769452* |
| *APOC1* | 19 | 2.59E−11 | 1.77E−02 | 3 | 3 | 0 | 3 | *rs439401*, *rs445925*, *rs4420638* |
| *TOMM40* | 19 | 1.35E−06 | 4.34E−02 | 3 | 3 | 1 | 2 | *rs157580*, *rs2075650*, *rs142412517* |
| *PGP* | 16 | 2.50E−06 | 8.90E−07 | 3 | 3 | 2 | 1 | *rs200526199*, *rs116977380*, *rs200615324* |
| *OTOL1* | 3 | 2.56E−04 | 7.11E−06 | 5 | 5 | 3 | 2 | *rs199791179*, *rs149127996*, *rs199985412*, *rs3921595*, *rs202021352* |
| *FN3KRP* | 17 | 9.19E−06 | 3.75E−02 | 5 | 5 | 3 | 2 | *rs138953335*, *rs61743692*, *rs144986629*, *rs142718764*, *rs1046896* |
| *SETD9* | 5 | 1.19E−05 | 6.95E−05 | 6 | 6 | 4 | 2 | *rs2257505*, *rs149334074*, *rs40497*, *rs141692637*, *rs150526244*, *rs146260337* |
| *RPS6KB1* | 17 | 1.56E−05 | 1.56E−05 | 2 | 2 | 1 | 1 | *rs201316437*, *rs1051424* |
| *GRN* | 17 | 1.59E−05 | 8.28E−03 | 4 | 4 | 4 | 0 | *rs63750723*, *rs63750043*, *rs63750541*, *rs63750412* |
| *PSG7* | 19 | 2.64E−05 | 4.19E−04 | 2 | 2 | 2 | 0 | *rs199532805*, *rs112354282* |
| *SKOR1* | 15 | 3.38E−05 | 9.20E−05 | 2 | 2 | 2 | 0 | *rs200956599*, *rs143419968* |
| *HNF4G* | 8 | 3.95E−05 | 2.17E−02 | 4 | 4 | 3 | 1 | *rs2943549*, *rs201625743*, *rs138897994*, *rs148532560* |
| *ASB17* | 1 | 4.42E−05 | 4.22E−05 | 3 | 3 | 1 | 2 | *rs149522654*, *rs11161887*, *rs3795251* |
| *SPZ1* | 5 | 8.09E−05 | 2.26E−04 | 5 | 5 | 2 | 3 | *rs1862136*, *rs139471643*, *rs184214819*, *rs200249535*, *rs35337118* |
| *BFSP1* | 20 | 1.25E−03 | 6.00E−05 | 6 | 6 | 5 | 1 | *rs145703098*, *rs140116733*, *rs6080719*, *rs147718368*, *rs143865632*, *rs142092768* |
| *HMHA1* | 19 | 1.54E−03 | 3.49E−05 | 8 | 8 | 5 | 3 | *rs1801284*, *rs2074442*, *rs36084354*, *rs142614852*, *rs150294461*, *rs139988914*, *rs61734935*, *rs139251906* |

*Notes*: Chromosome (Chr); P_skato and P_burden indicate the *p*-value calculated using SKAT-CommonRare function or the SKAT-O method, respectively, from R-package SKAT. All: number of SNVs genotyped for each gene set; Tested: all rare and common variants tested; Rare: number of rare variants tested. Common: number of common variants tested. NCBI: The National Center for Biotechnology Information. *APOE* = apolipoprotein E; *APOC1* = apolipoprotein C1; *ASB17* = ankyrin repeat and SOCS box containing 17; *BFSP1* = beaded filament structural protein 1; *FN3KRP* = fructosamine 3 kinase-related protein; *GRN* = granulin; *HMHA1* = Rho GTPase activating protein 45; *HNF4G* = hepatocyte nuclear factor 4 gamma; *OTOL1* = otolin 1; *PGP* = phosphoglycolate phosphatase; *PSG7* = pregnancy specific beta-1-glycoprotein 7; *RPS6KB1* = ribosomal protein S6 kinase B1; *SETD9* = SET domain containing 9; *SKOR1* = SKI family transcriptional corepressor 1; *SPZ1* = spermatogenic leucine.

seem to protect proteins from nonenzymatic glycation and stop the formation of certain advanced glycation end products (36). Strikingly, *rs1046896*-T, which was depleted in the LLI in our study (ORcond. = 0.77; ORcond. centenarians = 0.70), was identified as a risk locus for glycated hemoglobin (HbA1c), a critical nonenzymatic glycation product used to monitor and diagnose diabetes (39). Our findings, together with the substantial role of FN3KRP in cell maintenance and viability, support it as a promising candidate that may facilitate healthy aging and longevity.

In the German as well as the French samples, we observed a statistically significant association for *rs1063192* in the *CDKN2B-AS1* region. This particular SNV was previously suggested as part of a signature of exceptional longevity (11). Other *CDKN2B-AS1* SNVs, but not *rs1063192*, were described in (meta-)GWAS to be associated with longevity in various populations of European ancestry (12,13,40). Thus, our results support the relevance of this region, and maybe of *rs1063192* (or one of its LD SNVs), for the phenotype. The G-allele of *rs1063192* was enriched in our LLI relative to the controls (Table 2). Interestingly, this allele has been reported to be a protective variant in glaucoma, a classical age-related disease (41) that is also characterized by an increased burden of advanced glycation end products (38). Remarkably, we could not detect the *rs1063192* association in the Danish sample. The considerably smaller sample size (Danish: 1740 individuals; French: 3094 individuals; German 8189 individuals) as well as the older age of the Danish controls (<80.2 years; mean age: 66.3 years) compared with the German (<60 years) or French (<62 years) controls might have contributed to the failed replication. The lack of standardized criteria for the selection of suitable controls, together with varying phenotype definitions of longevity, has been suggested as one cause for discrepant association results (11,40,42).

In the single-variant analysis, we found exome-wide significant associations only for the 3 variants in the *TOMM40/APOE/APOC1* region (*rs2075650*, *rs4420638*, *rs769449*) and *rs1046896* (*FN3KRP*). Apart from these, 7 additional SNVs showed a suggestive association with longevity ($p < 2 \times 10E{-}04$, Table 1). Suggestively significant variants may still reflect true association signals with biological relevance, for example, it was shown that 8 of the 10 top not genome-wide significant CHARGE SNVs from a meta-analysis of GWA studies of longevity (25) corresponded to mouse life-span QTL (43). Of the 11 SNVs, for which we report a significant or suggestive longevity association, 4 are rare variants (MAF < 0.01, Table 1). In a recent study, in which the exomes of 100 LLI were sequenced, no rare protein-altering SNVs were observed to be enriched in the genomes of the LLI compared to younger controls (44). However, our results suggest that rare coding variants may very well be drivers of longevity.

Studies on other complex traits have shown that gene-based tests, in which the effects of all common and rare SNVs within a gene locus are considered jointly, can be more biologically relevant than single-variant association approaches. When we examined the cumulative effect of common and rare variants within each gene, we discovered *PGP* (cumulative effect of 3 variants: 2 rare and 1 common) as another novel longevity-associated locus ($p = 8.90 \times 10E{-}07$). Through its function as a glycerol-3-phosphate (Gro3P) phosphatase, PGP controls the levels of Gro3P that is an important metabolite formed during glycolysis (45). The availability of Gro3P is crucial for the regulation of both glucose and fat metabolism and eventually determines the generation of signaling and regulatory molecules which further affect many biological processes, for example, insulin secretion and sensitivity, inflammation, fat synthesis and storage, and (cancer) cell proliferation (46). Therefore, PGP may aid in the detoxification of excess nutrient/fuel supplies, thereby preventing metabolic stress and eventually facilitating longevity.

In the gene-based tests, apart from *PGP* and *TOMM40/APOE/APOC1,* we identified 12 additional potential longevity genes ($p < 1 \times 10E{-}04$), including otolin 1 (*OTOL1*, $p$-value < $1.61 \times 10E{-}06$ in the burden test). One intronic SNV in *OTOL1*, *rs1425609*, has already been reported in the context of longevity (47). The fact that the gene-based test also yielded a longevity association for *OTOL1*, which was first identified in a single-variant analysis (47), not only validates the gene-based approach, but also points towards the added value of analyzing both common and rare SNVs jointly for explaining the missing longevity heritability. Next to *OTOL1*, the 16 genes included the ribosomal protein S6 kinase B1 (*RPS6KB1*), a downstream effector of the nutrient-responsive mTOR (mechanistic target of rapamycin kinase) whose inhibition was shown to promote longevity in yeast, worms, and flies (48). Furthermore, a subset of 5 genes with suggestive statistical significance was involved in cell proliferation and cell growth (Supplementary Table 5). Interestingly, the gene *FN3KRP* reached a $p$-value of $3.08 \times 10E{-}06$ and survived multiple testing correction in the centenarian subset (Supplementary Table 5). This supports the *FN3KRP*-longevity association as a true-positive signal.

An accumulation of common and rare variants has already been observed in genes associated with other complex traits like type 2 diabetes (49) and Alzheimer's disease (50). With respect to extreme aging, the genes *LYST*, *MDN1*, and *RBMXL1* were found to harbor an increased burden of rare coding variants in LLI versus younger controls; however, with nominal significance only (44). To the best of our knowledge, the joint effect of common and rare variants on human longevity has not been investigated yet in a cohort of comparable size to ours. Recently, sets of variants with low frequency (MAF < 0.05) were analyzed for an association with longevity in 530 East Asian nonagenarians and centenarians. More than 100 genes reached nominal significance in that study. However, exome-wide significance was not met by any of the genes (17) and we could not identify any overlap between their and our results.

## Conclusion

Our study contributes to the genetic framework of longevity with 2 new potential candidate genes, *FN3KRP* and *PGP*, which were identified by single-variant and gene-based analyses, respectively. The 2 genes likely influence longevity through their role in metabolic processes, that is, the reverse glycation of proteins (*FN3KRP*) and control of Gro3P levels (*PGP*). However, with respect to *FN3KRP*, the variant that we report here (*rs1046896*) is unlikely to be the main causative variant, considering the high between-population heterogeneity values in the meta-analysis of the German, Danish, and French populations. Future fine-mapping studies are warranted to identify the true functional variant(s). With the combination of analysis methods and, in particular, the investigation of cumulative effects of common and rare variants within one genetic region, we are a small step closer to accounting for the missing heritability of human longevity.

## Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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## Author Contributions

G.G.T., J.D., F.F., A.F., and A.N. designed research; J.D., A.F., and A.N. supervised the project; W.L., A.F., F.F., D.E., S.S., K.S., M.M.-N., A.P., H.N., and P.H. were involved in recruitment of German study subjects and assembling of phenotypic data; F.F., D.E., and A.N. organized chip genotyping of German long-lived individuals; M.N., H.B., S.C., P.G., L.C., J.-F.D., and K.C. performed replication experiments; G.G.T. analyzed the data and together with A.C. performed the statistical analysis; G.G.T., J.D., and A.N. interpreted the data and wrote the manuscript; all authors performed critical revision and approved the final version of the manuscript.

## Conflict of Interest

None declared.

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