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Brief Report

Analysis of Polymorphisms in 59 Potential Candidate Genes for Association With Human Longevity

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Abstract

Longevity is a polygenic trait in which genetic predisposition is particularly important. We hypothesized that among genes differentially expressed in response to caloric restriction, several may be candidate longevity genes. We tested 459 single-nucleotide polymorphisms (SNPs) in 47 genes differentially expressed in calorically restricted mice and 12 other genes for association with longevity. Subjects were American men of Japanese ancestry, 440 aged ≥95 years and 374 with an average life span. Based on a dominant model of inheritance, an association with longevity at the $p < .05$ level was seen for SNPs in 13 of the genes. Testing by all possible models increased the number of genes to 18. After correction for multiple testing, four genes retained significance, namely, *MAP3K5* ($p = .00004$), *SIRT7* ($p = .00004$), *SIRT5* ($p = .0007$), and *PIK3R1* ($p = .01$). In a dominant model, association with longevity was seen for multiple adjacent SNPs within two of these genes (*MAP3K5* and *PIK3R1*), as well as in *FLT1*, consistent with linkage disequilibrium with a causative variant in the vicinity of each respective SNP set. *MAP3K5* and *FLT1* haplotypes were associated with longevity. In conclusion, the present study implicates variation in *MAP3K5*, *FLT1*, *PIK3R1*, *SIRT7*, and *SIRT5* in human longevity.

Keywords: Human genetics, Longevity, Quantitative genetics, Caloric restriction.

Genetic factors make a substantial contribution to attainment of extreme old age. Caloric restriction (CR) is well known to extend life span and delay age-related disease (1,2). Since variants that affect gene expression make a major contribution to the etiology of complex polygenic traits such as life span, we hypothesized that single-nucleotide polymorphisms (SNPs) of genes that undergo differential expression in response to CR in a mammalian model of human aging may be worthy of testing for association with human longevity. We noted a study by Estep et al. (3) that identified multiple genes differentially expressed in mouse liver in response to CR, 43 of the top 50 being listed in the GenAge mouse and human database. Upregulation was seen for genes encoding proteins involved in protein biosynthesis, rRNA processing, mRNA metabolism, pre-mRNA splicing, and regulation of protein translation (3). Downregulation was seen for genes encoding proteins involved in mitochondrial function, carboxylic acid metabolism, DNA replication, steroid, cholesterol and lipid metabolism, lysosome, peroxisome, and glutathione S-transferase activity. The majority of gene ontology (GO) categories applied to downregulated genes. Several downregulated GO classes were ones involved in protein turnover, including endoplasmic reticulum, isomerase activity, amino acid derivative biosynthesis, protein positioning, and the proteasome.

Since human longevity is associated with a depletion of metabolic pathways in a genotype-dependent manner (4), we were particularly

interested in testing SNPs in those genes for association with human longevity. Other genes revealed in the mouse CR study (3) included several involved in growth, stress resistance, and cell division, which we also considered worthy of testing.

The aim of our study was to test "tagging" SNPs (tSNPs) in human homologs of the genes identified in the mouse CR study for association with human longevity in a well-characterized population of American men of Japanese ancestry. Cases were 440 men who had lived to beyond 95 years of age and controls were 374 men recruited at the same time who had died at 78.1 ± 1.8 years of age (range 72–81 years). This cohort has proven to be successful for the discovery of SNPs in other genes with moderate effects on human longevity.

Methods

Study Cohort

See Supplementary Material.

Genotyping

For reasons of power, we included only common SNPs, i.e., those having a minor allele frequency (MAF) of ≥5% in the Japanese population as indicated by the JSNP database (http://snp.ims.u-tokyo.ac.jp) and with reference to the dbSNP/HapMap database (HapMap release 23a/phase II March 2008 on NCBI B36 assembly, dbSNP b126—http://www.ncbi.nlm.nih.gov/projects/SNP/). We included tSNPs in each gene plus 5 kb of 5′- and of 3′-flanking DNA. The reason for using tSNPs was to capture all genetic variation within the genes without having to assay every single variant. By interrogating a limited number of SNPs that are in linkage disequilibrium (LD) with one another it is possible to greatly reduce the total number of variants without jeopardizing discovery. For LD determination, the minimal coefficient of determination (r^2) value at which all alleles were to be captured was set to a threshold of 0.8 for the identification of all tSNPs.

Total leukocyte DNA was isolated using the PureGene system (Gentra Systems, Inc.) and quantified using PicoGreen staining (Molecular Probes, Eugene, OR). Initially, we tested 576 tSNPs from 58 genes, none of these tSNPs having been tested previously. The genes included 46 human homologs of the top 49 genes from Estep et al. (3) that exhibited statistically significant differential expression. These, and the expression change in CR mice (3), are shown in Table 1. We did not include *FOXO1* and *FOXO3* because we had published longevity-associations for SNPs in these 10 years earlier (5). We also genotyped tSNPs in 12 other genes of interest, namely *APOE/TOMM40*, *FAS*, *LMNA*, *RPTOR*, *RICTOR*, *SIRT1*, *SIRT2*, *SIRT3*, *SIRT4*, *SIRT5*, *SIRT6*, and *SIRT7*, of which *APOE*, *FAS*, *SIRT1*, and *SIRT5* were differentially expressed in Estep et al. (3), but not significantly so (see their Supplementary Table S2). tSNPs were genotyped at the University of Hawaii Cancer Center on the Illumina GoldenGate platform (high-throughput SNP genotyping on universal bead arrays (6)). Of the 576 tSNPs genotyped, 6 showed no variation, 5 were on the X-chromosome, and 51 failed to provide results, leaving 506 that were in Hardy–Weinberg equilibrium and 8 that failed to meet the Hardy–Weinberg equilibrium cut-off. In order to confirm our initial positive results and to fill gaps for assays that failed Illumina GoldenGate genotyping, we chose surrogates for SNPs that were in LD. Genotyping DNA from both cases and controls was performed on the same platform by Taqman. Surrogate SNPs were genotyped using allelic discrimination assays. TaqMan reagents (purchased from Applied Biosystems, Thermo Fisher Scientific) were used for PCR amplification under standard conditions with AmpliTaq Gold DNA polymerase (Perkin-Elmer Corp.). PCR products were detected by TaqMan assay, using a 6-FAM-labeled FRET probe for one allele and a VIC-labeled probe for the other allele, with minor groove binding quenchers to enhance assay signal. PCR products were measured using a QuantStudio 12K Flex system. Genotype data were managed through an integrated database system sample management-data processing system of proven accuracy (5). All positive controls on each genotyping plate were evaluated for consistency. Call rates for markers exceeded 98%.

We implemented a series of quality control checks based on the Illumina metrics. For inclusion of data for an SNP, its call rate had to exceed 0.95 and the Hardy–Weinberg equilibrium p value needed to be >.01.

Genes and SNPs Tested

The genes, location and number of SNPs in each that were tested are listed in Supplementary Table S1. We have previously reported

Table 1. Forty-Seven Human Homologs of the Top 49 Genes (in Alphabetical Order) From Estep et al. (3) and the Expression Change (±Fold) in Response to Caloric Restriction in Mouse Liver in That Study

Gene	±Fold change	Gene	±Fold change	Gene	±Fold change
APEX1	−1.3	*FOXO3*	+1.5	*NR3C1*	−1.5
APTX	−2.3	*GCLC*	−2.0	*PDPK1*	+1.3
AR	−2.8	*GCLM*	−1.5	*PIK3R1*	−1.4
ARHGAP1	−1.3	*GHR*	−2.1	*PLAU*	+2.7
BLM	+4.7	*GSTA4*	−1.7	*PPARA*	−2.3
CDKN1A	+10.2	*HSPA8*	+1.6	*PPARGC1A*	+6.0
CEBPA	−1.6	*IGFALS*	−2.4	*SGK1*	+4.6
CEBPB	+2.2	*JAK2*	−1.4	*SNCG*	−2.0
CEBPD	+4.3	*JUN*	+2.1	*STAT3*	+1.7
CTGF	+3.3	*LEPR*	+58.4	*TERT*	−2.9
CTSL	+4.9	*LMNB1*	−1.5	*TFDP1*	−1.3
DDIT4	+33.3	*MAP3K5*	+2.0	*TOP2A*	−1.5
EGFR	+1.7	*MAPK3*	−1.2	*TXN*	−1.5
ERCC3	−1.3	*MAPK9*	−1.3	*VCP*	−1.3
FLT1	+1.4	*NFKBIA*	+1.5	*XRCC5*	−1.3
FOXO1	+1.5	*NNMT*	+36.5		

the results of association analyses in our cohort for SNPs in *FOXO3* (5,7), *ATF4*, *CBL*, *CDKN2*, *EXO1* and *JUN* (8), *RICTOR*, *RPTOR* (except for two of the SNPs tested in the present study) and *RPS6KA1* (9), as well as *CTGF* and *EGFR* (10), so data for those genes are not presented in the current paper.

LD and Haplotype Mapping

Haplotype frequencies were calculated for the Japanese living in Tokyo data set using LDlink (11) and includes Phase 3 of the 1,000 Genomes Project, with SNP numbers indexed based on dbSNP build 142. This was assisted by online tools: https://analysistools.nci.nih.gov/LDlink. LD matrices were generated using the same tools. In our study, "haplotype" is the combination of specific alleles on a given stretch of DNA (i.e., a gene). Our expectation was that the minor alleles in a haplotype would be on a given strand of DNA. The use of haplotypes should have increased the specificity, resolution and accuracy of our genotype–phenotype comparisons since they were serving as surrogates for any functional variants in their respective neighborhoods.

Statistical Analyses

Genotype frequencies were evaluated for deviation from Hardy–Weinberg equilibrium in control samples. Differences between cases and controls were evaluated by Pearson χ^2 test and Fisher's exact test. As a first step, we evaluated our results from a biological perspective, asking the question: if the results for a SNP were real, rather than statistical "noise," then neighboring SNPs in LD should also show an association with the phenotype of interest, namely, longevity. Each of the genes found to contain longevity-associated variants satisfied these criteria in that there were several SNPs in LD that had similar MAF and p values for association with longevity.

For strength of association, odds ratios were estimated using logistic regression models. General linear models (GLM) were used to test for associations of continuous baseline variables with genotype according to the specific gene model, the t-test was used for dominant, recessive, and heterozygotes disadvantage models, and the trend test was used for the additive model. For continuous variables, the GLM was used. For the binary variables, the logistic model was used. All analyses were conducted using statistical software, SAS (12).

For genotype–phenotype comparisons, we initially used an "any minor allele" model since it captures the broadest category of individuals (e.g., "*AA*" vs. "*Aa*" + "*aa*", where "*A*" is the major allele and "*a*" is the minor allele). Once an association was found, we also utilized other models to determine if the significance would improve, including (i) additive ("*AA*" vs. "*Aa*" vs. "*aa*"), (ii) minor recessive ("*AA*" + "*Aa*" vs. "aa"), (iii) major recessive ("*AA*" vs. "*Aa*" + "*aa*"), (iv) heterozygote advantage ("*AA*" + "*aa*" vs. "*Aa*"), and (v) heterozygote disadvantage ("*AA*" + "*aa*" vs. "*Aa*"). For genotype–sub-phenotype comparisons, we used the best fit genetic model and phenotypes obtained in the longitudinal data of our cohort.

Results

Characteristics of Cases and Controls

The phenotypic data for cases (long-lived) and controls (normal life span) are shown in Supplementary Table S2. Many well-known risk factors were significantly lower in cases, namely fasting plasma glucose, fasting plasma insulin, plasma fibrinogen, white blood cell count, smoking history, difficulty walking 0.8 km, taking diabetes medication, coronary artery disease, stroke history, cancer, diabetes, emphysema, bypass history, angioplasty, cardiovascular surgery, ankle-brachial index. Various protective factors were higher in cases, namely expiratory volume, grip strength, cognitive score, and being married. Average year of birth for controls (1916.8) was 7 years later than cases (1909.9). After Bonferroni correction, cases showed significantly older age at examination, lower birth year, as well as higher forced expiratory volume, grip strength, ankle-brachial index, cognitive abilities screening instrument score, and physical activity index, but lower fasting plasma glucose, plasma fibrinogen concentration, white blood cell count, current smoking, pack-years smoking, alcohol consumption, difficulty walking 0.8 km, being on diabetes medication, coronary artery disease, stroke history, cancer, diabetes, aorta surgery, and ankle-brachial index <0.9.

Genetic Association with Longevity

The allele frequencies of the tSNPs in our control subjects were similar to those for Japanese in the dbSNP databank (Supplementary Table S3). We observed >98% concordance for all SNPs for HapMap samples that were assayed for quality assurance.

MAF of each of the SNPs for each gene in cases and controls, together with p value from χ^2 analysis testing each tSNP for association with longevity using an "any minor allele" model, are shown in Supplementary Table S4. Thirteen genes had tSNPs that showed a significant association with longevity at the $p < .05$ level (Table 2), after excluding *FOXO3*, *CTGF*, and *EGFR*, which we reported previously to contain tSNPs showing a significant association with longevity (5,7,10). Three genes—*FLT1*, *MAP3K5*, and *PIK3R1*—contained multiple tSNPs associated with longevity. These tSNPs were in LD, consistent with the expectation that we had captured sufficient genetic variation to include any functional or causal variants in their vicinities (i.e., on the block of conserved DNA). Haplotype analysis strengthened the significance for *MAP3K5* (haplotype *CT*; $p < .0001$) and *FLT1* (haplotype *CGC*; $p = .0005$), increasing the possibility that a causal variant for longevity would be captured in our analyses (Table 3). In contrast, haplotype analysis for the *PIK3R1* haplotype (*CAT*) was not significant.

Table 4 shows the results from applying multiple different models of inheritance to all genes. Statistical significance was noted for at least one tSNP in one model for 18 of the genes tested, the 5 additional genes being *SIRT4*, *STAT4*, *SIRT3*, *JAK2*, and *SIRT2*. After Bonferroni correction for multiple testing, the most statistically significant SNPs—in *MAP3K5*, *SIRT7*, *SIRT5*, and *PIK3R1*—remained significant ($p < .05$).

LD of SNPs

Supplementary Fig. S1 shows the locations of tSNPs that define the long-lived haplotype in *FLT1*, *GHR*, *JAK2*, *MAP3K5*, *MAP3K9*, *NR3C1*, *PIK3R1*, *RPTOR*, *SIRT1*, *SIRT2*, *SIRT3*, *SIRT4*, *SIRT5*, *SIRT7*, *STAT3*, *TERT*, *TFDP1*, and *TXN*. The longevity association of the tSNP(s) was defined by a dominant model (i.e. any minor allele) for all genes except *JAK2*, *SIRT2*, *SIRT3*, *SIRT4*, and *STAT3*.

Of particular note, for *MAP3K5*, *FLT*, and *PIK3R1* multiple tSNPs were associated with longevity. A block of LD is likely to contain a "functional variant" that modifies gene regulation, whereas the SNPs used in the current analyses were merely surrogates to indicate where a functional variant likely lies. Fine mapping of these regions by genotyping additional SNPs should help to improve the resolution in mapping longevity SNPs, as well as validating previous findings.

Table 2. Alphabetical List of Genes Showing Number of SNPs Tested in Each, Those SNPs That Were Associated with Longevity at the $p < .05$ Level Using a Dominant Model, and Those SNPs That Were Adjacent to Each Other

Gene	SNPs tested	Statistically significant SNP(s) and p value			Adjacent
FTL1	42	*rs3794396*	*rs7987649*	*rs9513099*	All 3 adjacent
		$P = .0007$	$p = .0189$	$p = .0250$	
GHR	14	*rs4130113*			
		$p = .0150$			
MAP3K5	34	*rs2076260*	*rs6904753*		Adjacent (in LD)
		$p = .0043$	$p = .0318$		
MAPK9	17	*rs7713083*			
		$p = .0135$			
NR3C1	18	*rs2963155*			
		$p = .0228$			
PIK3R1	19	*rs7709243*	*rs7713645*	*rs6881033*	Adjacent (in LD)
		$p = .0008$	$p = .0041$	$p = .0057$	
RPTOR	65	*rs9908495*			
		$p = .0114$			
SIRT1	4	*rs4746720*			
		$p = .0263$			
SIRT5	7	*rs2253217*			
		$p < .0001$			
SIRT7	2	*rs34829162*			
		$p = .0031$			
TERT	13	*rs2853676*			
		$p = .0159$			
TFDP1	5	*rs11839469*			
		$p = .0364$			
TXN	8	*rs3808888*			
		$p = .0051$			

Note. LD = linkage disequilibrium; SNP = single-nucleotide polymorphism.

Phenotype–Genotype Comparisons

Longevity was our single phenotype for this study. In an effort to provide information on specific organs or metabolic pathways involved in longevity in our cohort we chose to examine specific subphenotypes. Supplementary Table S5 shows the relationship of 40 phenotypes with genotypes of the most statistically significant tSNP in each of *MAP3K5*, *SIRT7*, *SIRT5*, *PIK3R1*, *FLT1*, *GHR*, *NR3C1*, *TERT*, and *TXN*. After Bonferroni correction, none remained significant at the $p < .05$ level. The Bonferroni correction may not be appropriate when determining significance for genes with modest effects on phenotype because statistical significance would be eliminated. Our previous results have shown that Bonferroni correction would have eliminated genotype–phenotype associations with SNPs in *FOXO3*, one of the most consistently replicated longevity genes, but which has only a modest effect on longevity (unpublished data). A more rational approach would be to promote SNPs that are either in close proximity and/or in LD with one another.

Table 3. Results of Haplotype Analysis for the 3 Genes Having Clustered Longevity-Associated tSNPs

Gene	SNP	*p* value
MAP3K5	*rs6904753-C*	.032
	rs2076260-T	.0043
	Haplotype: *CT*	<.0001
FLT1	*rs3794396-C*	.0007
	rs7987649-G	.019
	rs9513099-C	.025
	Haplotype: *CGC*	.00050
PIK3R1	*rs67527326-C*	.0041
	rs67529191-A	.0056
	rs67534039-T	.0008
	Haplotype: *CAT*	.58

Note. tSNP = tagging single-nucleotide polymorphism.

Discussion

The present study found possible support for an influence of genetic variation in *MAP3K5*, *SIRT7*, *SIRT5*, and *PIK3R1* on life span in American men of Japanese ancestry on the basis of the genetic model that showed a significant association with longevity and most statistically significant SNP of each. The approach used to identify longevity genes involved the use of a "tagging SNP" in which sentinel SNPs were used to capture all alleles with frequencies ≥.05 over the expanse of the gene plus 5 kb of 5′- and 3′-flanking DNA. A more detailed analysis would include additional SNPs in the vicinity of the "hits" in order to better validate associations. To date, there are no good methods for grouping statistical results into a more comprehensive *p* value. Rather, the use of Bonferroni correction is a method of reducing the risk of a chance association arising from multiple comparisons, and does not take into account the present situation of when multiple SNPs in a gene were in LD with one another. The latter should actually fortify an association. Three genes—*MAP3K5*, *PIK3R1*, and *FLT1*—contained multiple tSNPs that were associated with longevity and allowed haplotype analysis. Here, haplotype is referred to as a collection of alleles that appear in the same individual, and are compared to the reciprocal combination, since we have not performed detailed family studies to determine whether alleles are on the same strand of DNA (i.e., "genetic phase"). This added support to *MAP3K5* and *FLT1* being candidate longevity genes.

Table 4. Association of Tagging SNPs With Longevity in Those Genes Exhibiting $p < .05$

Gene	SNP	Genotype (refer)	Model	*OR* (95% *CI*)	*p*	Cases:controls
MAP3K5	*rs2076260*	*CT*,*TT* (*CC*)	Het disadvan	1.89 (1.41–2.54)	.0000025	393:344
SIRT7	*rs34829162*	*GG*,*TT* (*GT*)	Het disadvan	2.20 (1.52–3.18)	.0000025	371:352
SIRT5	*rs2253217*	*TT* (*TC*,*CC*)	Major recess	2.30 (1.54–3.42)	.000041	428:367
PIK3R1	*rs7709243*	*TT* (*TC*,*CC*)	Major recess	1.63 (1.22–2.18)	.00083	421:366
TERT	*rs2853677*	*AA*,*GG* (*AG*)	Het disadvan	1.66 (1.22–2.24)	.0011	379:350
SIRT4	*rs2522134*	*GG*,*AA* (*GG*)	Het disadvan	1.51 (1.14–2.01)	.0038	424:366
TXN	*rs3808888*	*GA*,*AA* (*GG*)	Minor dom	1.49 (1.13–1.96)	.0051	437:374
NR3C1	*rs9324921*	*CC*,*CA* (*AA*)	Major domin	1.87 (1.20–2.91)	.0056	438:374
RPTOR	*rs9908495*	*TT* (*TC*,*CC*)	Major recess	1.51 (1.10–2.07)	.011	419:346
MAP3K9	*rs7713083*	*TG*,*GG* (*TT*)	Minor domin	0.69 (0.52–0.93)	.014	440:374
STAT3	*rs4796791*	*CC*,*CT* (*TT*)	Major domin	1.50 (1.08–2.08)	.015	440:374
GHR	*rs4130113*	*AA* (*AG*,*GG*)	Major recess	1.43 (1.07–1.91)	.015	440:374
SIRT3	*rs11246009*	*AA*,*TT* (*AT*)	Het disadvan	1.44 (1.06–1.96)	.019	415:362
FLT1	*rs2296190*	*GG*,*CG*,*CC*	Additive	1.49 (1.05–2.10)	.024	420:366
SIRT1	*rs4746720*	*TT* (*TC*,*CC*)	Major recess	1.41 (1.01–1.92)	.026	408:356
JAK2	*rs3824432*	*AA* (*AG*,*GG*)	Minor recess	2.79 (1.10–7.07)	.030	433:371
SIRT2	*rs10405150*	*CC* (*CT*,*TT*)	Minor recess	3.95 (1.12–14.0)	.033	409:364
TFDP1	*rs11839469*	*GC*,*CC* (*GG*)	Minor domin	1.55 (1.03–2.33)	.036	440:374

Note. Definitions of genetic models: heterozygote disadvantage = major and minor allele homozygotes at an advantage for longevity; heterozygote advantage = heterozygotes at an advantage; major recessive = major allele homozygotes at an advantage; minor recessive = minor allele homozygotes at an advantage; major dominant = one or two major alleles at an advantage; minor dominant = one or two minor alleles at an advantage; additive = codominant so that homozygotes for the minor allele have an advantage over heterozygotes. Genes are listed in order of strength of statistical significance of the most significant SNP and the most favorable model. This list does not include those SNPs identified by the dominant model shown in Table 2. However, some SNPs were significant for more than one model. *p* values shown were from χ^2 analysis; "refer" is the reference genotype for the model tested. *CI* = confidence interval; OR = odds ratio; SNP = single-nucleotide polymorphism.

For typical traits, the strongest SNPs explain only a modest fraction of the predicted genetic variability, with analyses showing that common SNPs with modest *p* values, such as those that became nonsignificant after Bonferroni correction, may nevertheless contribute to longevity-associated heritability (13). For example, *FOXO3* is now well-accepted as a robust longevity gene (14,15), despite modest *p* values having been found in our initial report (5). Such modest *p* values would be eliminated by Bonferroni correction, as is commonly used in genome-wide association studies (GWAS). We will now discuss how the four genes, *MAP3K5*, *SIRT7*, *SIRT5*, and *PIK3R1*, might contribute to the phenotype of longevity.

Most of the genes chosen for our study were found to be differentially expressed in mouse liver in response to CR (3). This raises the possibility that genetic variants we found to be associated with longevity might do so by influencing the expression of the gene where they are located or may be in LD with specific causative variant(s) that influence expression of that gene.

Mitogen-activated protein kinase kinase kinase 5 (MAP3K5, also termed ASK1) is a member of a family of enzymes involved in kinase-signaling cascades in the cell. It is involved in cell differentiation and survival, apoptosis, innate immune response, and oxidative stress response, all of which confer increased survival. Owing to the multiple SNPs in LD and significant haplotype results, results point to *MAP3K5* as the most likely longevity candidate among all of the genes we analyzed in the present study. Klotho, a suppressor of aging, downregulates the ASK1-signalosome (a ROS-sensitive complex) to reduce p38 MAPK activity and senescence pathways, thereby promoting longevity (16). Variants in this region of the gene are reported to be associated with expression level, insulin resistance, and type 2 diabetes in Pima Indians (17).

Our haplotype analysis also implicated the Fms-related tyrosine kinase gene (*FLT1*) in longevity. *FLT1* codes for the vascular endothelial growth factor 1 receptor, which is involved in stimulation of vasculogenesis and angiogenesis, so helping to maintain blood supply to tissues. In the CR mouse study, *Flt1* was upregulated (3). Interestingly, the major differentially expressed gene in the mouse CR study was the DNA damage-inducible transcript 4 gene (*Ddit4*) (3). DDIT4 regulates cell growth, proliferation and survival by inhibition of mechanistic target of rapamycin complex 1 in response to cellular energy levels and stress, including responses to reactive oxygen species, hypoxia, and DNA damage. Although SNPs in *DDIT4* were not associated with longevity, DDIT4 is in the FLT1 transmembrane tyrosine kinase receptor pathway. The *FLT1* SNP *rs7987649* and four others in proximity to it are reported to influence colorectal cancer survival (18). Our study found *rs2296190* in *FLT1* was associated with cancer (Supplementary Table S5).

After testing SNPs in all seven sirtuin genes, we found SNPs in *SIRT7* and *SIRT5* to be the ones most strongly associated with longevity. Sirtuins have important functions in cell homeostasis and processes that affect healthy aging and longevity (19). *SIRT7* SNPs have not previously been found to be associated with human longevity. Sirtuin 7, located in the nucleolus, binds to multiple nuclear and nucleolar proteins, many involved in regulation of RNA polymerase I transcription, increases resistance to oxidative stress, maintains mitochondrial number, and promotes stem cell survival (19,20). In the case of *SIRT5*, an association between a SNP and longevity has been reported previously (21). Sirtuin 5 is located in mitochondria where it has an important role in metabolism, in particular detoxification of ammonia (19). Sirtuin 5 is cardioprotective (22). Differential methylation of the promoters of both *SIRT5* and *SIRT7* occur in aging (19). The *SIRT1* SNP *rs4746720* we found to be weakly associated with longevity in dominant and recessive models ($p = .014$ and $p = .023$ before correction for multiple testing) was associated with longevity in Chinese (23). Associations with longevity have been reported for SNPs in other sirtuin genes, where, using other models, we found weak association with longevity for SNPs in *SIRT2*, *SIRT3*, and *SIRT4* ($p = .033$, .019, and .0038) before correction for multiple testing.

Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) is the regulatory subunit of phosphoinositide-3-kinase in the insulin

signaling cascade. Downregulation of insulin signaling is associated with increased life span. Although we noted an association of genetic variation in *PIK3R1* with longevity, a negative result was obtained in a GWAS of nonagenarians (24). The *PIK3R1* SNP *rs7713645* has been reported to be associated with body fat and serum leptin levels (25), and may modify the effect of body size and weight gain on breast cancer risk (26). In our study, the three significant SNPs were associated with weight, BMI, and forced expiratory volume in 1 second.

Conclusion

In this, the first study to assess the human homologs of genes that in mice exhibit the strongest differential expression in response to caloric restriction, we tested SNPs in 47 such genes, as well as 12 other genes, and found SNPs in 13 to be associated with longevity using a dominant model and 5 more genes when using other models in our cohort of American men of Japanese ancestry. After excluding three genes (*FOXO3*, *CTGF*, and *EGFR*) that we reported previously as being associated with longevity and applying Bonferroni correction, we found that SNPs in *MAP3K5*, *SIRT7*, *SIRT5*, and *PIK3R1* remained statistically significant. *MAP3K5*, *PIK3R1*, *and FLT1* contained multiple longevity-associated tSNPs in close proximity to each other within each of these genes and haplotype analysis of these tSNPs implicated variation in *MAP3K5* and *FLT1* in longevity. The longevity-associated alleles of the tSNPs we identified may be in LD with the allele of regulatory variant(s) that have a stronger effect on gene expression that the alternative allele, so boosting the levels of the encoded protein and leading to higher beneficial biological effects for the specific protein.

Supplementary Material

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

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Conflict of Interest

None reported.

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