THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Medical Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2017, Vol. 72, No. 10, 1411–1416
doi:10.1093/gerona/glx011
Advance Access publication February 18, 2017

OXFORD

Research Article

Genomewide Association Scan of a Mortality Associated Endophenotype for a Long and Healthy Life in the Long Life Family Study

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Received May 18, 2016; Editorial Decision Date January 5, 2017

Decision Editor: Stephen Kritchevsky, PhD

Abstract

Background: Identification of genes or fundamental biological pathways that regulate aging phenotypes and longevity could lead to possible interventions to increase healthy longevity.

Methods: Using data from the Long Life Family Study, we performed genomewide association analyses on an endophenotype construct, LF1, comprising a linear combination of traits across health domains. LF1 primarily reflected traits from the pulmonary and physical activity domains.

Results: We detected a significant association between LF1 and a locus on chromosome 10p15 (p-value = 4.65×10^{-8}) and suggestive evidence (p-value < 5×10^{-6}) for association on chromosomes 1, 2, 8, 12, 15, 18, and 22. Using data from the Health, Aging and Body Composition Study, we subsequently replicated the association for the 1p13 region near the *NBPF6* locus (p-value = 3.65×10^{-4}).

Conclusions: Our analyses indicate that loci influencing a healthy aging endophenotype construct predominantly comprised of pulmonary and physical function domains may be located on chromosome 1p13 near the *NBPF6* locus. Further investigation of this possible locus and other suggestive loci may reveal novel biological pathways that influence healthy aging.

Keywords: Respiratory function—Motor activity—Mortality—Human genetics—Longevity

Identification of genes or biological pathways that influence longevity and healthy aging could enable the development of interventions that will increase functional longevity, and thus decrease some of the age-related burden of disease. Studies in animal models, such as the nematode *Caenorhabditis elegans*, have revealed several genes that have dramatic effects on longevity (1). Additional genes with strong effects on longevity have been reported in yeast, flies, and mice (2–4). Although longevity is known to be heritable in humans (5), identification of specific genes that influence longevity in humans has been challenging (6). Numerous linkage, candidate gene, and genomewide association (GWA) studies have been performed for longevity in humans, but except for *APOE*, *FOXO3*, and a locus on chromosome 3, the results of these studies have been inconsistent (reviewed by Brooks-Wilson (6)). A GWA study of longevity in CHARGE Consortium including 6,036 cases (≥90 years) and 3,757 controls that died between ages 55 and 80 years also confirmed the association of *FOXO3* and *APOE* with longevity (7). Meta-analysis of longevity GWA studies of long-lived individuals of European descent identified a novel locus on chromosome 5q33.3 and also replicated the association of *TOMM40/APOE/APOC1* locus (8).

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Recently, Flachsbart and colleagues (9) reported association of a novel longevity locus on chromosome 5q31.1. One factor that may be limiting new discovery is the heterogeneity of longevity. Potentially, healthy aging traits using disease free survival or combinations of health measures could be more specifically associated with genetic factors.

We previously developed a novel approach to define healthy aging phenotypes in the Long Life Family Study (LLFS) (10). Using factor analysis, five heritable traits or endophenotypes comprised of linear combinations of 28 traits across five health domains: cognition, cardiovascular, metabolic, physical activity, and pulmonary were established (10). Of these five heritable endophenotypes, the most dominant endophenotype, LF1, primarily reflected the physical activity and pulmonary domains. In addition, LF1 was independently and significantly associated with lower mortality (p-value < 10^{-6}) and also attenuated 24.1% of the effect of increased age on mortality in LLFS (11). We subsequently validated this approach in a second cohort, the Health, Aging and Body Composition cohort (Health ABC). The most dominant endophenotype in the Health ABC cohort, HF1, predominantly reflected the physical activity and pulmonary domains, was significantly and independently associated with decreased mortality (p-value < 10^{-10}), and attenuated the effect of age by 18.9% (11). Thus, this endophenotype construct identified a latent, heritable, healthy aging trait related to longevity. In the current study, we report the results of GWA analyses of the LF1 endophenotype construct in LLFS, and subsequent replication in the Health ABC cohort with the HF1 endophenotype construct.

Methods

Study Design and Subjects

LLFS is a family-based cohort recruited by four study sites across the United States and Denmark. Family ascertainment has been described previously (12). Briefly, sibships were selected based on the exceptionality of the sibship's survival into old age using a Family Longevity Selection Score (12). In addition, we recruited the offspring of each member of the long-lived sibship, and the offspring's and sibling's spouses. For the current study, complete phenotypic and genotypic data were available on 3,876 participants from 568 families. Development of the endophenotype construct was described previously (10,11). Endophenotype values for individuals were calculated using traits with factor loadings ≥ |0.3|.

Phenotypic and genotypic data were available on 1,470 European American individuals from Health ABC, a longitudinal study of healthy men and women between the ages of 68 and 80 at baseline. Characteristics of the LLFS and Health ABC cohorts and factor loadings of the endophenotype construct (called LF1 in LLFS and HF1 in Health ABC) are given in Table 1. As can be seen, the factor loadings for each trait in LF1 and HF1 are similar, despite the absence of cognitive domain traits in Health ABC.

Genotyping and Imputation for Association Analysis

The Center for Inherited Disease Research (CIDR) assayed all LLFS subjects using the Illumina Human Omni 2.5 v1 chip. Quality control was performed by CIDR and the LLFS Coordinating Center. Single-nucleotide polymorphisms were excluded if they had low call rate (<98%), had Mendelian errors, or Hardy–Weinberg equilibrium deviations (p-value < 10^{-6}). To account for population substructure, principal components were estimated (EIGENSTRAT) (13) using genotypes on 1,522 unrelated LLFS individuals. These principle components for ancestry were expanded to all family members. Imputed genotypes were generated based on the cosmopolitan phased haplotypes of 1000 Human Genome (1000HG, version 2010/2011 data freeze, 2012/2003/2004 haplotypes) using MACH (version 1.0.16, for pre-phasing of LLFS data) and MINIMACH (version of May 2012) (14,15). This process led to a hybrid dataset with 38,245,546 SNPs, of which 2,225,338 SNPs were genotyped and 36,020,208 SNPs were imputed. Prior to the association analyses, additional filters were used (removing monomorphic SNPs, MAF < 1% and imputation quality score of r^2 < .3), that reduced the number of variants for analysis from 38.25 M to 9.25 M.

In Health ABC, genotyping was performed by CIDR using the Illumina Human1M-Duo BeadChip system. SNPs with MAF < 1%, with call rate < 97% and HWE p-value < 10^{-6} were removed. Imputation was done using 1.2 million successfully genotyped SNPs and 1,663 subjects using the 1000 Genomes reference haplotypes (June 2010 release). Additional information on genotyping, imputation, and derivation of principle components for ancestry have been previously reported (16).

Statistical Analysis and Bioinformatic Analyses

GWA scan was performed in LLFS assuming additive effects using a linear mixed-effect regression model correcting for family structure (17). LF1 was adjusted for sex, age, recruitment site and ancestry principle components by including these covariates in the model. Thresholds for suggestive and significant levels of association were p-value < 5×10^{-6} and p-value < 5×10^{-8}, respectively. Prior to initiating the study, LLFS investigators performed extensive power calculations indicating that we have 80% power to detect tag SNPs for latent causal variants that account for ≥ 1%–2% of the total endophenotypic variance.

To select an independent set of SNPs for replication, approximate conditional analyses were performed using GCTA software (18) at each suggestive locus (p-value < 5×10^{-6}) selected for replication. Analyses were conducted by including all the SNPs within a window of ±1 MB around the lead SNP by using stepwise selection method (19) and independent SNPs (defined as having p-value < 10^{-5}) were selected for follow-up in Health ABC. Association analyses were performed in the Health ABC cohort using ProbABEL (ProbABEL v. 0.4.1) (20). The following covariates were included in the models to test for association with the HF1 endophenotype: age, sex, recruitment center, and ancestry principle components.

For the subset of the SNPs that were selected for replication in Health ABC, combined p-values were also calculated using the fixed-effect inverse variance approach as implemented in the METAL package (21).

Functional annotation of the candidate SNPs was investigated using RegulomeDB (22). As the most significant SNP in a region may not be the functional SNP, proxy SNPs (r^2 threshold ≥ .8) were identified using SNP Annotation and Proxy Search (SNAP; https://archive.broadinstitute.org/mpg/snap) and also included in the analysis. We also performed pathway analysis by first mapping all SNPs to the RefSeq gene regions (±5 kb from the gene boundaries); a total of 10,947 SNPs with p-value < .001 were mapped to 1,116 genes. The lowest p-value of the SNPs within the gene region was assigned as the significance value for the gene. These genes were then imported into Ingenuity Pathway Analysis tool to assess whether specific canonical pathways were significantly enriched (IPA; http://www.ingenuity.com/).

Results

Overall, the GWA scan for LF1 revealed evidence of association for a total of 72 SNPs, from suggestive loci, on chromosomes 1,

Table 1. Population Characteristics of Persons and the Factor Loadings of the Endophenotype Construct in the Long Life Family Study (2006–2009) and the Health, Aging and Body Composition Study (1997–1998)

	LLFS (n = 3,876)	Health ABC (n = 1,470)	Factor Loading	
Characteristics	Mean (*SD*) or Frequency (%)	Mean (*SD*) or Frequency (%)	LF1 Factor Loadings	HF1 Factor Loadings
Age, years	67.93 ± 14.57	73.68 ± 2.83		
Female sex	55%	48%		
Cognitive domain				
Animal recall test[a]	20.46 ± 6.36	na	0.19	na
Vegetable recall test[a]	14.04 ± 4.66	na	−0.11	na
Digit span forward test[b]	8.30 ± 2.19	na	0.05	na
Digit span backward test[b]	6.47 ± 2.27	na	0.05	na
Immediate memory test[c]	12.15 ± 4.44	na	0.00	na
Delayed memory test[c]	10.58 ± 4.83	na	0.00	na
Cardiovascular domain				
Hypertension[d] (yes/no)	50%	43%	−0.04	−0.05
Systolic blood pressure, mm Hg	130.77 ± 20.88	133.15 ± 18.98	−0.02	−0.05
Diastolic blood pressure, mm Hg	77.51 ± 10.99	69.91 ± 10.84	0.22	0.19
Pulse pressure, mm Hg	53.12 ± 16.79	63.24 ± 16.31	−0.18	−0.18
Total cholesterol, mg/dL	201.00 ± 41.55	201.20 ± 36.30	−0.08	−0.26
HDL cholesterol, mg/dL	59.29 ± 17.15	52.44 ± 15.79	−0.29	−0.43
LDL cholesterol, mg/dL	119.38 ± 35.24	119.88 ± 32.29	0.03	−0.04
Triglyceride, mg/dL	108.47 ± 57.58	144.35 ± 66.64	0.03	−0.09
Metabolic domain				
Diabetes[e] (yes/no)	6%	9%	−0.16	−0.08
Body mass index[f]	27.14 ± 4.66	26.46 ± 4.08	0.04	0.09
Creatinine, mg/dL	1.02 ± 0.24	1.00 ± 0.22	0.34	0.50
Fasting glucose, mg/dL	93.98 ± 15.62	98.56 ± 20.94	−0.04	0.03
Glycosylated hemoglobin, %	5.58 ± 0.45	6.05 ± 0.69	−0.19	−0.09
Waist circumference, cm	94.59 ± 13.71	98.65 ± 11.61	0.19	0.21
Physical activity domain				
Average grip strength, kg	29.36 ± 11.62	29.26 ± 9.77	0.88	0.86
Maximum grip strength, kg	30.21 ± 11.84	32.04 ± 10.36	0.88	0.85
Gait speed, m/s	1.07 ± 0.29	1.25 ± 0.22	0.45	0.45
Physical performance[g]	10.41 ± 2.52	10.37 ± 1.35	0.44	0.39
Pulmonary domain				
Lung disease[h] (yes/no)	12%	14%	−0.14	−0.20
FEV1, mL	2493.83 ± 852.09	2291.47 ± 653.11	0.86	0.84
FEV6, mL	3227.02 ± 1031.71	2994.89 ± 800.91	0.87	0.87
FEV1:FEV6, %	76.98 ± 6.83	76.50 ± 7.31	0.09	0.08

Notes: FEV1 = forced expiratory volume in 1 s; FEV6 = forced expiratory volume in 6 s; HDL = high-density lipoprotein; Health ABC = Health, Aging, and Body Composition; LDL = low-density lipoprotein.

[a]Score: Number of items the participant can name in 60 s. [b]Source: Wechsler Memory Scales—III. Range of possible scores, 0–12. [c]Source: Wechsler Memory Scales—Revised. Range of possible scores, 0–25. [d]Hypertension was defined as a self-report of hypertension confirmed by the use of antihypertensive medication or systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. [e]Diabetes was defined as use of diabetes medication or fasting glucose concentration ≥126 mg/dL. [f]Weight (kg)/height (m)^2. [g]Physical performance was the sum of results from chair stands, a set of balance tests, and walking performance on a short-distance walk. Units could range from 0 to 12. [h]Lung disease was defined as a self-report of a previous diagnosis of chronic bronchitis, emphysema, or chronic obstructive pulmonary disease.

2, 8, 10, 12, 15, 18, and 22 with suggestive p-values < 5×10^{-6} (Supplementary Figure 1 and Supplementary Table 1); one locus on chromosome 10p15 reached genomewide significance. The variant with the strongest association (p-value = 4.65×10^{-8}) is an insertion/deletion (indel), that is located 27 kb downstream of the *KLF6* gene. To select SNPs for replication, conditional analyses were performed at 12 suggestive genomic loci as described in the methods. We did not detect any additional signals in these regions, so the 12 lead SNPs from these regions were chosen for replication in the Health ABC cohort. A Bonferroni-corrected p-value ≤ .004 (0.05/12) was considered to be significant evidence for replication.

There was no replication of the locus on chromosome 10p15 (near *KLF6*) in Health ABC; that is, endophenotype HF1 showed no association with any of the tested SNPs in this region (Table 2). The 10p15 region may be a false positive or it may be a true association and the causal variant is in linkage disequilibrium with tagging SNPs in our novel LLFS cohort. However, there was a significant association (Health ABC p-value = 3.65×10^{-4}, combined p-value = 1.67×10^{-9}) between endophenotype HF1 and the locus on 1p13; an indel that is located 16 kb upstream of the *NBPF6* gene (Table 2). A locus in the 1q42 region also reached genome-wide significance in the combined meta-analysis, (combined p-value = 1.17×10^{-8}); SNP rs4477283 is situated about 62 kb downstream of *CAPN9* (Table 2). In addition, the locus on 18q11, reached suggestive genome-wide significance in the combined analyses (replication p-value = .054, combined p-value = 4.86×10^{-7}). In single SNP analyses, these SNPs accounted for 0.7%–1.0% of the variation.

Table 2. Results for Replication of QTLs for Endophenotype 1 in the Health, Aging and Body Composition Study

SNP	Region	Position (build 37)	Minor/Major Allele	MAF	Candidate Genes (within 60 kb)	LLFS Beta	LLFS *p*-value	Health ABC Replication		Combined *p*-value
								Beta	*p*-value	
rs201856309	**1p13.3**	**108901784**	**D/R**	**0.026**	***NBPF6; NBPF5***	**−1.182**	**4.04E-06**	**−1.238**	**3.65E-04**	**1.67E-09**
rs677325	1q31.2	191510125	A/C	0.132		0.434	9.64E-07	−0.050	.672	2.14E-04
rs4477283	**1q42.2**	**230932442**	**T/C**	**0.203**	***CAPN9; C1orf198***	**−0.361**	**6.63E-07**	**−0.269**	**.005**	**1.17E-08**
rs116083259	2p22.1	41090895	C/G	0.015		−1.484	3.34E-06	−0.789	.076	9.18E-07
rs2346184	2q23.3	153407553	T/C	0.366	*FMNL2*	−0.290	2.52E-06	0.039	.627	4.60E-04
rs4675226	2q33.1	202632938	T/C	0.276	*ALS2; CDK15*	0.319	1.29E-06	−0.020	.822	1.69E-04
rs77980484	8q24.12	120221376	G/T	0.017	*MAL2*	1.352	1.39E-07	−0.114	.731	5.89E-05
rs61019025	10p15.2	3790907	D/R	0.327	*KLF6*	0.367	4.65E-08	0.005	.954	8.13E-06
rs73687059	12q21.31	84015550	A/G	0.048		−0.787	5.10E-07	0.085	.764	2.08E-05
rs1284049	15q24.1	73120343	G/A	0.473	*ADPGK*	−0.269	3.76E-06	0.113	.148	.005
rs7237853	18q11.2	22984635	T/C	0.147	*ZNF521*	0.405	1.49E-06	0.221	.054	4.86E-07
rs2568457	18q21.2	49147023	G/A	0.247		−0.311	4.56E-06	0.137	.132	.003

Notes: Health ABC = Health, Aging, and Body Composition; LLFS = Long Life Family Study; QTLs, Quantitative Trait Loci.
* *p*-values (Health ABC replication) ≤ .005 are highlighted in bold.

We next used RegulomeDB (22) to investigate whether any of the significant or proxy SNPs across the three regions of interest might be functional, but the scores of these SNPs were not indicative of function (RegulomeDB score ≥ 5) or data were unavailable. Results of the initial pathway analyses revealed that the most significant canonical pathway was the ubiquitous calcium transport I pathway (five genes/10 total genes in the pathway; p-value = 6.38×10^{-5}). When the analysis was restricted to SNPs with p-value < 10^{-5} (only 13 genes were included), the Amyotrophic Lateral Sclerosis signaling pathway was the most significantly enriched pathway (two genes *ALS2* and *CAPN9* out of 111 total genes in the pathway, p-value = 2.06×10^{-3}).

Discussion

In the current study, using GWA analysis on the LF1 endophenotype construct in LLFS, we obtained suggestive or significant evidence for association with loci in several chromosomal regions (Supplementary Figure 1). We then tested 12 SNPs across these regions for replication using data from the Health ABC cohort, and obtained a significant evidence for association of an indel in chromosomal regions 1p13 (upstream of the *NBPF6* gene). Two other SNPs, rs4477283 (1q42; Health ABC p-value = .005) and rs7237853 (18q11; Health ABC p-value = .054) also reached nominal significance levels respectively. In the combined meta-analysis, the locus on 1q42 region reached genome-wide significance and the 18q11.2 region was suggestive.

NBPF6 (1p13) is a member of the neuroblastoma breakpoint family (NBPF). *NBPF* genes contain numerous repetitive elements and are abundantly expressed in breast and liver tissues (23). Calcium dependent protease *CAPN9* (1q42) is predominantly expressed in the digestive tract (24) and also part of the Amyotrophic Lateral Sclerosis signaling pathway (25). *ZNF521* (18q11) is expressed in multiple tissues (including brain, heart, skeletal muscles, spleen, and hematopoietic cells). The ZNF521 protein is a transcription factor, containing 30 kruppel-like zinc fingers and has been shown to play a role in erythroid cell differentiation (26,27).

The LF1 and HF1 endophenotype constructs primarily reflect physical function and pulmonary health in the LLFS and Health ABC cohorts, respectively. Both physical function and pulmonary health decline at older ages, perhaps because of a decline in skeletal muscle strength (28). Thus, the relationship between the identified loci and the endophenotype construct is unclear. These results may indicate that a novel neuromuscular biological pathway is involved in healthy aging and/or skeletal muscle strength. Alternatively, these associations may be spurious or the associated SNPs are not marking variation in these regions, but may be marking another, as yet unidentified function in these chromosomal regions.

Over the past decade, investigators have performed association studies (both candidate gene and genomewide) on long-lived individuals to identify loci that may contribute to "desirable phenotypes," such as longevity and healthy aging. Most of the results of these studies using data from long-lived individuals have been inconsistent, perhaps indicating the environmental and genetic complexity, such as heterogeneity, that underlies longevity. The best replicated findings are for variation at the *APOE* locus and *FOXO3* (7,29). The effects of variation at *APOE* on longevity are well-replicated and well-known (30–32). The *FOXO3* gene lies within the insulin/insulin-like growth factor 1 signaling pathway, a pathway that is known to extend lifespan in several animal models. We assessed whether variants in the 5q33, *FOXO3*, and *APOE* loci were associated with the LF1 endophenotype. The LF1 endophenotype was not associated

with any of the variants, with the exception of a nominal association with the *APOE* e4 allele (*p*-value = .026) (results not shown). This result is not surprising given that longevity is a complex, heterogeneous trait. Furthermore, LF1 was predominantly comprised of traits from the pulmonary and physical activity domain, and not the metabolic function domain traits that have been associated with *FOXO3* variants (33,34). Fewer association studies of healthy aging phenotypes have been performed, partly because healthy aging has been defined in various ways, including the absence of various disease or morbidities at a pre-defined "older" age (such as event-free survival) or the presence of desirable traits, such as mobility at a specific "older" age. To date, none of them have reported genomewide significant results (35,36).

This study has several limitations. One limitation of the current study is the sample size, which reduces our power to detect rare variants. However, power calculations indicated that we have 80% power to detect tag SNPs for latent causal variants that account for >1%–2% of the total endophenotypic variance. Another limitation is that families in LLFS were recruited using a score based on exceptional longevity in families, thus identification of comparable cohorts for replication is challenging. The Health ABC cohort, a study of healthy individuals recruited between ages 68 and 80 years old, is a reasonable replication cohort, but identification of additional cohorts is necessary. In addition, multiple traits across different domains were used to derive the endophenotype, and relatively few studies have data on all these traits. However, only a subset of traits contributed substantially to the endophenotype, and this smaller subset is likely to be available in additional studies.

Our analyses indicate that loci influencing a healthy aging endophenotype construct predominantly comprised of pulmonary and physical function domains may be located on chromosome 1p13 near the *NBPF6* locus, chromosome 1q42.2 near *CAPN9*, and perhaps, 18q11.2 near *ZNF521*. Further investigation of these novel loci may reveal additional biological pathways that fundamentally influence healthy aging.

Supplementary Material

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

Funding

This work was supported by the National Institute on Aging at the National Institutes of Health (LLFS: U01AG023712, U01AG023744, U01AG023746, U01AG023749, and U01AG023755; Health ABC: AE-2-1024, N01-AG-6-2101, N01-AG-6-2103, and N01-AG-6-2106).

Acknowledgments

The authors wish to thank the participants in LLFS and Health ABC who made this study possible.

Conflict of Interest

None declared.

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