

# GWAS of Longevity in CHARGE Consortium Confirms *APOE* and *FOXO3* Candidacy

Linda Broer,<sup>1,2,3</sup> Aron S. Buchman,<sup>4</sup> Joris Deelen,<sup>2,5</sup> Daniel S. Evans,<sup>6</sup> Jessica D. Faul,<sup>7</sup>  
Kathryn L. Lunetta,<sup>8,9</sup> Paola Sebastiani,<sup>8</sup> Jennifer A. Smith,<sup>10</sup> Albert V. Smith,<sup>11,12</sup>  
Toshiko Tanaka,<sup>13</sup> Lei Yu,<sup>4</sup> Alice M. Arnold,<sup>14</sup> Thor Aspelund,<sup>11,12</sup> Emelia J. Benjamin,<sup>9,15,16</sup>  
Philip L. De Jager,<sup>17,18,19</sup> Gudny Eiriksdottir,<sup>11</sup> Denis A. Evans,<sup>20</sup> Melissa E. Garcia,<sup>21</sup>  
Albert Hofman,<sup>1,2</sup> Robert C. Kaplan,<sup>22</sup> Sharon L. R. Kardia,<sup>10</sup> Douglas P. Kiel,<sup>18,23,24</sup>  
Ben A. Oostra,<sup>1,2</sup> Eric S. Orwoll,<sup>25</sup> Neeta Parimi,<sup>6</sup> Bruce M. Psaty,<sup>26,27,28,29</sup> Fernando Rivadeneira,<sup>1,2,3</sup>  
Jerome I. Rotter,<sup>30</sup> Sudha Seshadri,<sup>8,31</sup> Andrew Singleton,<sup>32</sup> Henning Tiemeier,<sup>1,2,33</sup>  
André G. Uitterlinden,<sup>1,2,3</sup> Wei Zhao,<sup>10</sup> Stefania Bandinelli,<sup>34</sup> David A. Bennett,<sup>35</sup>  
Luigi Ferrucci,<sup>13</sup> Vilmundur Gudnason,<sup>11,12</sup> Tamara B. Harris,<sup>21</sup> David Karasik,<sup>23,36</sup>  
Lenore J. Launer,<sup>21</sup> Thomas T. Perls,<sup>37</sup> P. Eline Slagboom,<sup>2,5</sup> Gregory J. Tranah,<sup>6,38</sup> David R. Weir,<sup>7</sup>  
Anne B. Newman,<sup>39,\*</sup> Cornelia M. van Duijn,<sup>1,2,\*</sup> and Joanne M. Murabito<sup>9,40,\*</sup>

<sup>1</sup>Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands.

<sup>2</sup>Netherlands Consortium for Healthy Ageing, Leiden University Medical Center, The Netherlands.

<sup>3</sup>Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands.

<sup>4</sup>Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, Illinois.

<sup>5</sup>Department of Molecular Epidemiology, Leiden University Medical Center, The Netherlands.

<sup>6</sup>California Pacific Medical Center Research Institute, San Francisco.

<sup>7</sup>Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor.

<sup>8</sup>Department of Biostatistics, Boston University School of Public Health, Massachusetts.

<sup>9</sup>NHLBI's and Boston University's Framingham Heart Study, Massachusetts.

<sup>10</sup>Department of Epidemiology, University of Michigan, Ann Arbor.

<sup>11</sup>Icelandic Heart Association, Kopavogur, Iceland.

<sup>12</sup>Department of Medicine, University of Iceland, Reykjavik.

<sup>13</sup>Translational Gerontology Branch, National Institute on Aging, Baltimore, Maryland.

<sup>14</sup>Department of Biostatistics, University of Washington, Seattle.

<sup>15</sup>Department of Medicine, Sections of Preventive Medicine and Cardiology, Boston University School of Medicine, Massachusetts.

<sup>16</sup>Department of Epidemiology, Boston University School of Public Health, Massachusetts.

<sup>17</sup>Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women's Hospital, Boston, Massachusetts.

<sup>18</sup>Harvard Medical School, Boston, Massachusetts.

<sup>19</sup>Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts.

<sup>20</sup>Rush Institute for Healthy Aging and Department of Internal Medicine, Rush University Medical Center, Chicago, Illinois.

<sup>21</sup>Laboratory of Epidemiology and Population Sciences, National Institute on Aging, Bethesda, Maryland.

<sup>22</sup>Department of Epidemiology and Population Health, Albert Einstein College, Bronx, New York.

<sup>23</sup>Institute for Aging Research, Hebrew SeniorLife, Harvard Medical School Department of Medicine, Boston, Massachusetts.

<sup>24</sup>Division of Gerontology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts.

<sup>25</sup>School of Medicine, Oregon Health and Science University, Portland.

<sup>26</sup>Department of Medicine, University of Washington, Seattle.

<sup>27</sup>Department of Epidemiology, University of Washington, Seattle.

<sup>28</sup>Department of Health Services, University of Washington, Seattle.

<sup>29</sup>Group Health Research Institute, Group Health Cooperative, Seattle, Washington.

<sup>30</sup>Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute and Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California.

<sup>31</sup>Department of Neurology, Boston University School of Medicine, Massachusetts.

<sup>32</sup>Laboratory of Neurogenetics, National Institute on Aging, Bethesda, Maryland.

<sup>33</sup>Department of Child and Adolescent Psychiatry, Erasmus MC and Sophia Children's Hospital, Rotterdam, The Netherlands.

<sup>34</sup>Geriatric Unit, Azienda Sanitaria Firenze, Florence, Italy.

<sup>35</sup>Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, Illinois.

<sup>36</sup>Faculty of Medicine in The Galilee, Bar-Ilan University, Safed, Israel.

<sup>37</sup>Section of Geriatrics, Boston University School of Medicine and Boston Medical Center, Massachusetts.

<sup>38</sup>Department of Epidemiology and Biostatistics, University of California, San Francisco.

<sup>39</sup>Department of Epidemiology, University of Pittsburgh, Pennsylvania.

<sup>40</sup>Department of Medicine, Section of General Internal Medicine, Boston University School of Medicine, Massachusetts.

\*These authors contributed equally to this work.

Address correspondence to Joanne Murabito, Boston University School of Medicine, Framingham Heart Study, 73 Mount Wayte, Suite 2, Framingham, MA 01701. Email: [murabito@bu.edu](mailto:murabito@bu.edu)

**Background.** The genetic contribution to longevity in humans has been estimated to range from 15% to 25%. Only two genes, *APOE* and *FOXO3*, have shown association with longevity in multiple independent studies.

**Methods.** We conducted a meta-analysis of genome-wide association studies including 6,036 longevity cases, age  $\geq 90$  years, and 3,757 controls that died between ages 55 and 80 years. We additionally attempted to replicate earlier identified single nucleotide polymorphism (SNP) associations with longevity.

**Results.** In our meta-analysis, we found suggestive evidence for the association of SNPs near *CADM2* (odds ratio [OR] = 0.81;  $p$  value =  $9.66 \times 10^{-7}$ ) and *GRIK2* (odds ratio = 1.24;  $p$  value =  $5.09 \times 10^{-8}$ ) with longevity. When attempting to replicate findings earlier identified in genome-wide association studies, only the *APOE* locus consistently replicated. In an additional look-up of the candidate gene *FOXO3*, we found that an earlier identified variant shows a highly significant association with longevity when including published data with our meta-analysis (odds ratio = 1.17;  $p$  value =  $1.85 \times 10^{-10}$ ).

**Conclusions.** We did not identify new genome-wide significant associations with longevity and did not replicate earlier findings except for *APOE* and *FOXO3*. Our inability to find new associations with survival to ages  $\geq 90$  years because longevity represents multiple complex traits with heterogeneous genetic underpinnings, or alternatively, that longevity may be regulated by rare variants that are not captured by standard genome-wide genotyping and imputation of common variants.

**Key Words:** Longevity—GWAS—FOXO3—APOE.

Received March 28, 2014; Accepted August 7, 2014

Decision Editor: Stephen Kritchevsky, PhD

THERE is ample evidence that genetic factors are involved in extreme longevity both in humans and in other organisms. In model organisms, ranging from *Caenorhabditis elegans* to *Mus musculus*, mutations in the insulin/IGF-1 signaling (IIS) pathway have been shown to substantially increase lifespan (1–6). Other suggested mechanisms involve stress resistance as mediated by heat shock proteins (7,8), cellular senescence as mediated by telomere length (9) and inflammation/immune function (10). The genetic contribution to longevity in humans has been estimated to range from 15% to 25% (11–13).

Although many human candidate longevity genes have been investigated, only two genes have been widely replicated. The first consistent association reported was *APOE* (14). It was subsequently replicated in both candidate gene and genome-wide association studies (GWAS) with genome-wide significant evidence (15–20). The second longevity gene, identified in American male centenarians of Japanese descent and replicated in other candidate gene studies that included both men and women across ethnicities, was *FOXO3*, although this gene was not identified in GWAS (21–25).

Early GWAS have failed to identify novel longevity genes as reviewed by Murabito and coworkers (26). In 2010, the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium published a meta-analysis of 1,836 longevity (90+) cases and a comparison group of 1,955 individuals who died between 55 and 80 years of age (27). A total of 24 independent SNPs were identified, though none reached genome-wide significance. Finally, a recent GWAS by Deelen and coworkers identified one new longevity locus on 5q33.3 (28).

The key to success to GWAS has proven to be large sample sizes. Since the original CHARGE longevity GWAS (27) additional members of each cohort have reached or exceeded age of 90 years and additional studies have joined the consortium, permitting an expanded meta-analysis of longevity. Using the much expanded sample of 6,036 longevity cases and 3,757 controls and the same study design as the original CHARGE longevity study, we attempted to produce robust new associations and replicate earlier identified associations for longevity coming from several earlier studies.

## METHODS

### Participants

The participants in our study were of European ancestry and included cohorts from the CHARGE Consortium (29). All cohorts periodically assess the vital status of their participants. Although some of the cohorts include multiple ethnic groups, only data from Caucasian participants were used. Informed written consent was obtained from all participants, and appropriate institutional approvals were obtained. A brief description of each population is given in the [Supplementary Material](#).

### Longevity Phenotype

Longevity was defined as reaching age  $\geq 90$  years. A more extreme longevity threshold of age 100 years was considered. However, the sample size was deemed insufficiently powered. Genotyped participants who died between the

ages of 55 and 80 years were used as the control group. The control group was limited to deceased participants to ensure that no control individuals could subsequently achieve longevity. The minimum age at death was set to match the minimum age at enrollment in the Rotterdam Study. The maximal age at death was set arbitrarily at the age of 80 years to include the majority of deaths and to exclude those participants who survived far beyond the average life expectancy for their respective birth cohort. Across the discovery cohorts, there were 6,036 participants who achieved longevity and the control group had 3,757 participants.

We did not have direct replication cohorts; however, we performed a look-up of our strongest associations in three independent studies with similar phenotypes, one of which is a published GWAS on longevity (30).

### Genotyping and Imputation

As different genotyping platforms were used across studies, we imputed to ~2.5 million SNPs using the HapMap 22 CEU (Build 36) genotyped samples as a reference. Details on the study-specific quality control procedures for genotyping and imputation are found in [Supplementary Table 1](#). Although genotyping between different studies was performed on different genotyping platforms, genotyping of cases and controls within studies was performed on the same genotyping platform in each study.

### Statistical Analysis

We used logistic regression to test each SNP for association with longevity using an additive model adjusting for sex and principal components to adjust for population stratification. Fixed-effects inverse-variance meta-analysis was performed using METAL. The  $p$  values were corrected for genomic control. Associations with a  $p$  value  $< 5 \times 10^{-8}$  were considered genome-wide significant, whereas associations with a  $p$  value  $< 1 \times 10^{-5}$  were considered suggestive and taken forward to the look-up phase.

Detailed analysis of cohorts participating in the look-up phase and additional analyses can be found in the [Supplementary Material](#). Conditional analysis of the *FOXO3* locus was performed using the GCTA tool which analyzes aggregate, not individual level data (31,32). We further attempted to replicate earlier identified SNP associations with longevity. Finally, we used gene networks available at [www.genenetworks.nl](http://www.genenetworks.nl) as a bioinformatics resource to further investigate significant findings.

## RESULTS

General characteristics of discovery and look-up cohorts are found in [Tables 1](#) and [2](#), respectively. Between 0.0% and 70.3%, varying per cohort, of those achieving longevity were still alive at the time that longevity status was ascertained. Among those who had died, the distribution of

causes of death differed between longevity cases and the comparison group. While 1.0%–17.3% of those achieving longevity died of cancer, 10.1%–50.3% of deaths in the control group could be attributed to cancer.

None of the SNP-longevity associations reached the genome-wide significance threshold of  $5 \times 10^{-8}$  in the discovery phase ([Supplementary Figure 1](#)), although the strongest association was borderline significant for rs1416280 located 369 kb from *GRIK2* (odds ratio [OR] = 1.24;  $p$  value =  $5.09 \times 10^{-8}$ ). In total, seven loci passed the threshold for suggestive association ( $< 1 \times 10^{-5}$ ) and were included in the look-up phase ([Table 3](#)). For look-up, we also included the most significant SNP in the current study from the known candidate longevity gene, *FOXO3* ( $p$  value =  $8.56 \times 10^{-5}$ ). Forest plots of all eight SNPs can be found in [Supplementary Figure 2](#). Although none of the SNPs reached Bonferroni adjusted significance in any of the look-up cohorts, consistent results considering direction of effect were found for *FOXO3* across discovery and look-up cohorts ([Table 3](#)). Association results for *CADM2* and, to a lesser degree, *GRIK2* were consistent across cohorts. In pathway analysis using the gene networks tool, both *CADM2* and *GRIK2* genes were found to be involved in neuronal pathways ([Supplementary Table 2](#)).

Of the 24 SNPs identified as suggestively associated in the original CHARGE longevity GWAS (27), the  $p$  value for only one of these (*GRIK2*; rs954551) improved in the current study ([Supplementary Table 3](#)). One additional region (*RGS7*) from the earlier study appeared among the strongest associations in the current study, but the association was not strengthened in the look-up phase of this study.

Next, we aimed to replicate the GWAS of Sebastiani and coworkers conducted on centenarians ([Supplementary Table 4](#)) (20). Two hundred and eighty-one SNPs were reported using four different genetic models (20). Six out of the 281 SNPs were reported to have a  $p$  value  $< 1 \times 10^{-5}$  in the additive model. We attempted to replicate these six SNPs, and only the SNP at the *APOE* locus replicated at the Bonferroni corrected significance level (OR = 1.20;  $p$  value =  $4.8 \times 10^{-4}$ ). Similarly, of the four regions identified with linkage analysis of longevity sib-pairs (age  $\geq 90$  years) (33) we also replicated only the *APOE* locus ([Supplementary Table 5](#)).

Finally, in a candidate gene approach, we examined the original SNP reported for the *FOXO3* gene (rs2802292) in the current study and added our current study (each discovery cohort individually) to the published studies reporting on the same *FOXO3* SNP ([Supplementary Table 6](#)) (21–25,30). In the current study, rs2802292 reached a  $p$  value of .012 (OR = 1.09). When including the published studies, both candidate gene and GWAS, in the meta-analysis rs2802292 reached a  $p$  value of  $1.85 \times 10^{-10}$  (OR = 1.17). The Linkage Disequilibrium (LD) as measured by  $r^2$  between this SNP and the top *FOXO3* SNP in the current study (rs10457180) was .64. In conditional analyses of the discovery phase

Table 1. General Characteristics of Discovery Cohorts

		N	Age at DNA Draw			Cause of Death (%)*			
			Mean (SD)	Women (%)	Alive (%)	CVD	Cancer	Other	Unknown
Rotterdam Study 1 (RS1)	90+ cases	899	82.3 (6.1)	79.0	23.6	31.4	7.9	60.7	0.0
	Comparison	1,192	66.5 (5.6)	41.3	0.0	32.2	40.1	27.7	0.0
Rotterdam Study 2 (RS2)	90+ cases	69	86.0 (6.2)	60.9	69.6	33.3	14.3	52.4	0.0
	Comparison	161	66.0 (3.3)	37.3	0.0	23.6	50.3	26.1	0.0
Study of Osteoporotic Fractures (SOF) <sup>†</sup>	90+ cases	1,720	72.7(5.8)	100.0	54.4	16.8	3.6	21.5	0.0
	Comparison	124	69.6 (3.4)	100.0	0.0	37.9	28.2	33.9	0.0
Cardiovascular Health Study (CHS)	90+ cases	791	77.7 (5.0)	62.3	41.7	51.5	17.3	30.6	0.6
	Comparison	560	69.5 (3.0)	53.2	0.0	33.6	39.3	27.0	0.2
Osteoporotic Fractures in Men Study (MrOS) <sup>‡</sup>	90+ cases	670	83.0 (3.4)	0.0	43.6	19.9	6.9	21.8	3.1
	Comparison	502	70.8 (3.4)	0.0	0.0	29.3	38.8	31.7	0.2
Framingham Heart Study (FHS)	90+ cases	320	86.7 (4.3)	66.9	0.0	24.1	11.6	51.9	12.5
	Comparison	484	65.4 (7.3)	34.3	0.0	24.6	43.8	25.2	6.4
Health and Retirement Study (HRS)	90+ cases	384	89.3 (3.1)	67.5	68.5	3.0	15.0	12.6	69.5
	Comparison	401	69.4 (6.2)	45.6	0.0	12.1	10.1	16.2	61.6
Age, Gene/Environment SusceptibilityReykjavik Study (AGES)	90+ cases	541	84.9 (2.8)	61.2	65.1	27.3	8.4	16.2	48.1
	Comparison	145	72.8 (3.0)	49.0	0.0	28.2	35.5	18.4	18.0
Religious Orders Study and Rush Memory and Aging Project (RADC)	90+ cases	468	85.9 (5.0)	75.2	40.0	0.0	0.0	0.0	100.0
	Comparison	78	70.2 (3.4)	48.7	0.0	0.0	0.0	0.0	100.0
Invecchiare nel Chianti (InCHIANTI)	90+ cases	101	92.8 (2.9)	72.3	70.3	8.9	3.0	7.9	80.2
	Comparison	75	72.8 (4.0)	36.0	0.0	6.7	20.0	4.0	69.3
Baltimore Longitudinal Study of Ageing (BLSA)	90+ cases	128	91.42 (3.96)	49.2	50.0	4.7	1.0	3.0	91.4
	Comparison	42	69.14 (6.25)	26.2	0.0	9.5	19.0	2.3	69.1

Notes: \*In cases the % of all deaths is reported.  
<sup>†</sup> In SOF, the vital status is not known for all individuals.  
<sup>‡</sup> In MrOS, the vital status is not known for all individuals.

Table 2. General Characteristics of Look-Up Cohorts

	NECS		LLFS	EU_longevity
	Cases (100+)	Comparison	Survival Analysis	Cases (90+); Comparison (<65)
N	801	914	4,567	5,409; 16,121
Age at DNA draw (median [range])	104 (95–119)	73 (54–90)	67 (25–110)	NA
Follow-up (median [range])		NA	3 (0–7)	NA
Women, %	72.0	56.0	55.0	NA
Alive, %	3.0	0.0	83.0	NA

Notes: LLFS = Long Life Family Study; NECS = New England Centenarian Study. No study specific information for the EU\_longevity consortium was available. Causes of death were not available in the look-up cohorts.

cohorts, including both SNPs, only rs10457180 remained significant at nominal *p* value (OR = 0.94; *p* value = 5.53 × 10<sup>-4</sup>).

**DISCUSSION**

In this investigation of 6,036 longevity cases and 3,757 controls, we found suggestive evidence for the involvement of SNPs near *CADM2* and *GRIK2*. We further confirmed the associations of *APOE* and *FOXO3* with longevity.

*FOXO3* is a known candidate gene for longevity and part of the well-characterized IIS pathway (6). Although originally identified in candidate gene studies, it did not earlier reach genome-wide significance (21–25). In this study, the strongest signal in the *FOXO3* gene was found for rs10457180 which is in LD (*r*<sup>2</sup> = .64) with the earlier

identified SNP rs2802292. Pooling our data with that of independent studies from literature yielded a *p* value of 1.85 × 10<sup>-10</sup>. Although we included all studies reporting on *FOXO3* association with longevity from the literature as well as contacted authors of earlier published GWAS for information on *FOXO3* in their study (19), publication bias, which is common in candidate gene studies, may be influencing the results of this analysis. In a conditional analysis of the discovery phase cohorts, only rs10457180 remained significant, which suggests that rs10457180 may be a better tagging SNP for the true causal variant. A point of notice is that *FOXO3* has 99% sequence homology in its exonic regions with its pseudogene (*ZNF286B*), which could complicate the calling of genetic variants for this gene (34,35). However, the investigated variants (rs2802292 and

Table 3. Association Results for Longevity: Discovery and Look-Up

SNP	Chr	Gene	Distance (kb)	EA	EAF	Discovery			EU_longevity			Centenarian (NECS)			Survival (LLFS)				
						p Value	OR	95% CI	Direction	effect	p Value	r <sup>2</sup> *	OR	p Value	r <sup>2</sup> *	SNP	r <sup>2</sup> *	I/HR	p Value
rs1416280	6	<i>GRIK2</i>	369	C	0.75	5.09 × 10 <sup>-8</sup>	1.24	1.15–1.34	+++++	+	.276	rs9377361	1.00	0.98	.833	rs9377361	1.00	1.00	.981
rs9841144	3	<i>CADM2</i>	-236	A	0.79	9.66 × 10 <sup>-7</sup>	0.81	0.74–0.88	-----+	+	.825	rs9822731	0.96	1.03	.726	rs9822731	0.96	0.85	.053
rs4611001	1	<i>RG57</i>	-28	A	0.97	1.84 × 10 <sup>-6</sup>	1.79	1.41–2.27	+?+++++	+	.207	rs7536260	0.68	0.65	<b>.013</b>	rs75361849	1.00	1.07	.728
rs11023737	11	<i>SOX6</i>	-28	A	0.32	3.64 × 10 <sup>-6</sup>	0.83	0.77–0.90	-----+	+	.684	rs2196961	0.27	1.03	.668	rs11023744	0.96	1.06	.397
rs11753077	6	<i>MBOAT1</i>	-76	T	0.64	7.51 × 10 <sup>-6</sup>	1.17	1.09–1.26	+++++	+	.095	rs7763815	0.87	1.04	.572	Same	1.00	1.03	.633
rs10875746	12	<i>PFKM</i>	Intron	A	0.76	7.83 × 10 <sup>-6</sup>	1.20	1.11–1.30	+++++	+	.240	rs2228500	0.74	1.03	.796	rs2228500	0.74	0.93	.349
rs10007810	4	<i>LIMCH1</i>	Intron	A	0.23	8.80 × 10 <sup>-6</sup>	1.20	1.11–1.30	+++++	-	.979	Same	1.00	0.98	.841	Same	1.00	0.95	.521
rs10457180	6	<i>FOXO3</i>	Intron	A	0.70	8.56 × 10 <sup>-5</sup>	0.87	0.81–0.93	-----+	-	<b>.023</b>	rs2153960	0.89	0.98	.822	rs2153960	0.89	0.84	<b>.021</b>

Notes: EA = effect allele; EAF = effect allele frequency; “+” = effect allele over-represented in cases; “-” = effect allele under-represented in cases; “?” = not tested in this cohort. The p values < .05 in replication are represented in bold. EU\_longevity: European consortium investigating the genetics of longevity with similar phenotype to discovery (cases: 90+; controls: < 65 y old); Centenarians (NECS, New England Centenarian Study); centenarian cases compared to younger controls; Survival (LLFS, Long Life Family Study): all-cause mortality Cox-regression analysis, I/HR shown for direct comparison of the direction of the effect. In NECS and LLFS, imputation is not available. Study order for *direction* column: RS1, RS2, SOF, CHS, MrOS, FHS, HRS, AGES, RADDC, InCHIANTI, BLSA.

\*Linkage disequilibrium with discovery SNP.



rs10457180) are both intronic, which are not present in *ZNF286B*. Therefore, we believe our results are not affected by this phenomenon.

Although our look-up for both *CADM2* and *GRIK2* did not show consistent associations, these genes are interesting candidate genes for longevity. Both genes are involved in neuronal pathways, in particular in neuron cell–cell adhesion and regulation of glutamatergic synaptic transmission ([www.genenetworks.nl](http://www.genenetworks.nl)). Neuronal pathways have been implicated earlier in all-cause mortality in a formal pathway analysis (36).

We did not replicate other earlier reported associations with longevity, except for *FOXO3* and *APOE*. Although many attempts to unravel the genetic contribution to the longevity phenotype have been undertaken, it has proven difficult to find robust associations (26). Genome-wide association studies have identified the genetic architecture of many complex traits with great success, even when the heritability of the trait is modest, as is the case for longevity. Yet the longevity phenotype remains elusive to uncovering new genetic associations.

As with any chosen methodology GWAS has its pitfalls. The most obvious pitfall of a GWAS is the low  $p$  value of significance required to call a variant significantly associated. There are many potential sources of Type I and II errors in GWAS, including case–control misclassification, nongenetic covariates, and population stratification, which can only partly be accounted for in the design of the study (37). We have used the standard approach of considering  $p$  values  $< 5 \times 10^{-8}$  as significant to prevent false positive findings. As a result, we accept the presence of false negative findings. Recently, we have shown that the number of false positive findings increases exponentially when higher  $p$  values are used in GWAS setting, further justifying the use of a stringent cut-off (38). We do not believe that case–control misclassification is a big issue in our study as we have very clear cut-offs for being included as a case ( $\geq 90$  years) or control (deceased between 55 and 80 years). The potential influence of nongenetic covariates is more difficult to ascertain. However, these are assumed to be independent of genotypes and are therefore unlikely to be confounders. In addition, it has been shown that the power of meta-analysis is not optimal for SNPs with small to modest effect sizes (39). However, it is common practice in GWAS to perform large-scale meta-analyses as the sample size required to detect variants at such low  $p$  values is not sufficient in any one study. Increasing the sample size of the current study may help identify new variants.

There are several potential explanations for why longevity has proven difficult to dissect genetically. First, the longevity phenotype simply could be too complex and heterogeneous for successful identification of longevity genes. Achieving longevity may require a great number of “protective” genes all with small effects (20). Although in highly heritable traits, like height, genes with very small

effects have been uncovered, in a trait like longevity it may be challenging to identify individual genetic variants given the numbers of available genotyped individuals reaching longevity. In addition, the complexity of the longevity phenotype is made more challenging due to its interaction with a number of age-related diseases, that is, cancer, cardiovascular disease, and dementia, all of which have independent genetic risk variants. Although it has been shown that currently identified variants for age-related diseases do not strongly associate with longevity (40), suggesting that there are longevity-specific variants remaining to be discovered, this genetic and phenotypic heterogeneity of longevity makes it very challenging to identify genetic variants for longevity.

Using biomarkers that explain (a part of) longevity might be a more fruitful pursuit for finding associations with longevity itself. Unfortunately, no good biomarkers of aging currently exist, although many have been proposed (41). Telomere length, a marker of senescence, could be an interesting biomarker of aging and, GWAS have already proven successful in identifying genes for this trait (42). An alternative approach could be the development of novel phenotypes like the healthy aging index, which incorporates information across physiologic systems and predicts mortality better than age itself (43).

The second potential reason for not finding any genome-wide significant associations with the longevity phenotype is that GWAS targets common variants. As longevity in the general population is quite rare, but common in specific long-lived families, it could be that rare variants, rather than common variants are involved in the longevity phenotype (44). Such variants are not likely to be picked up in GWAS, but may be uncovered with rare variant association analysis, which requires sequencing technology (45).

Another avenue that deserves consideration for future studies is the identification of joint genetic effects (epistasis) (46). Epistasis has been demonstrated to be an important contributor to age-related diseases such as cardiovascular disease (47). Methods for identifying epistasis are improving and becoming feasible for genome-wide studies (48).

Finally, it is possible that epigenetic changes could influence the expression of genetic variations leading to longevity. Epigenetic modifications such as DNA methylation and histone modification are essential for development and differentiation, but can also arise later in life (49). Age-related changes in DNA methylation have been implicated in senescence and longevity (50). Such changes may be a major contributor to longevity that might make it difficult to find associations with common genetic variants. A known phenomenon in longevity is that the majority of individuals reaching longevity are female. Whether this has a genetic or epigenetic basis is unknown and should be investigated in future studies.

A limitation of our phenotype is our definition of longevity, namely, living to age  $\geq 90$  years may not be “extreme” enough as a case definition. We considered more extreme

longevity cut-offs, including centenarian status; however, we found that the sample size for such currently was not enough to have sufficient power to detect association in GWAS. Similarly, the definition of dying before 80 years might be too liberal for the definition of controls since they survived far beyond the average life expectancy for their respective birth cohort. Furthermore, more extreme cut-offs would have resulted in a too small sample size for GWAS.

In conclusion, we confirmed the association of *FOXO3* and *APOE* with longevity, but did not find any new associations, aside from the suggestive evidence for *CADM2* and *GRIK2*. Notably, variants in neuronal pathway genes were confirmed to play a role in longevity. Future genetic studies should consider: (i) using more extreme age definitions for longevity, (ii) performing rare variant association analyses, (iii) analyzing epistasis, (iv) exploring epigenetic changes associated with longevity, (v) sex-specific analysis, and (vi) increasing the sample sizes of both cases and controls.

#### SUPPLEMENTARY MATERIAL

Supplementary material can be found at: <http://biomedgerontology.oxfordjournals.org/>

#### FUNDING

The Rotterdam Study is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project nr. 050-060-810, Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

SOF is supported by National Institutes of Health funding. The National Institute on Aging (NIA) provides support under the following grant numbers: R01 AG005407, R01 AR35582, R01 AR35583, R01 AR35584, R01 AG005394, R01 AG027574, and R01 AG027576.

This CHS research was supported by NHLBI contracts HHSN 268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the National Institute on Aging (NIA). A full list of CHS investigators and institutions can be found at <http://www.chs-nhlbi.org/pi.htm>. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

MrOS is supported by National Institutes of Health funding. The following institutes provide support: the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Center for Research Resources (NCRR), and NIH Roadmap for Medical Research under the following grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AG027810, and UL1 RR024140. NIAMS provides funding for the MrOS ancillary study 'Replication of candidate gene associations and bone strength phenotype in MrOS' under the grant number R01-AR051124. NIAMS provides funding for the MrOS ancillary study 'GWAS in MrOS and SOF' under the grant number RC2ARO58973.

The Framingham Heart Study genetic association analyses for this project were supported by the National Institute of Aging (R01AG29451,

J.M. M., K.L.L.). "This research was conducted in part using data and resources from the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. This work was partially supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center." E.J.B. and KLL were funded by R01AG028321 and R01HL092577. D.P.K.'s effort was supported by a grant from the National Institute of Arthritis, Musculoskeletal and Skin Diseases and the NIA (R01 AR/AG 41398).

HRS is supported by the National Institute on Aging (NIA U01AG009740). The genotyping was funded separately by the National Institute on Aging (RC2 AG036495, RC4 AG039029). Our genotyping was conducted by the NIH Center for Inherited Disease Research (CIDR) at Johns Hopkins University. Genotyping quality control and final preparation of the data were performed by the Genetics Coordinating Center at the University of Washington.

AGES is funded by NIH contract number N01-AG-12100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). Genotyping was conducted at the NIA Intramural Research Program Laboratory of Neurogenetics.

The MAP and ROS data used in this analysis was supported by National Institute on Aging grants P30AG10161, R01AG17917, R01AG15819, R01AG30146, the Illinois Department of Public Health, and the Translational Genomics Research Institute.

The InCHIANTI study baseline (1998–2000) was supported as a "targeted project" (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336).

The BLSA was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

The analysis of LLFS and NECS data were supported by the National Institute on Aging (NIA cooperative agreements U01-AG023755 and U19-AG023122 to T.P.)

#### ACKNOWLEDGMENTS

Cohort specific acknowledgements can be found in the [Supplementary Material](#).

#### REFERENCES

- Bohni R, Riesgo-Escovar J, Oldham S, et al. Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1-4. *Cell*. 1999;97:865–875. doi:S0092-8674(00)80799-0 [pii]
- Clancy DJ, Gems D, Harshman LG, et al. Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science*. 2001;292:104–106. doi:10.1126/science.1057991 292/5514/104 [pii]
- Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology*. 2000;141:2608–2613.
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature*. 1993;366:461–464. doi:10.1038/366461a0
- Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science (New York)*. 1997;277:942–946.
- Libina N, Berman JR, Kenyon C. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell*. 2003;115:489–502. doi:S0092867403008894 [pii]
- Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science*. 2008;319:916–919.
- Gething MJ, Sambrook J. Protein folding in the cell. *Nature*. 1992;355:33–45.

9. Shay JW, Wright WE. Senescence and immortalization: role of telomeres and telomerase. *Carcinogenesis*. 2005;26:867–874. doi:10.1093/carcin/bgh296
10. Jeck WR, Siebold AP, Sharpless NE. Review: a meta-analysis of GWAS and age-associated diseases. *Aging Cell*. 2012;11:727–731. doi:10.1111/j.1474-9726.2012.00871.x
11. Herskind AM, McGue M, Holm NV, Sørensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum Genet*. 1996;97:319–323.
12. McGue M, Vaupel JW, Holm N, Harvald B. Longevity is moderately heritable in a sample of Danish twins born 1870–1880. *J Gerontol*. 1993;48:B237–B244.
13. Mitchell BD, Hsueh WC, King TM, et al. Heritability of life span in the old order Amish. *Am J Med Genet*. 2001;102:346–352. doi:10.1002/ajmg.1483
14. Schachter F, Faure-Delanef L, Guenet F, et al. Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet*. 1994;6:29–32. doi:10.1038/ng0194-29
15. Bathum L, Christiansen L, Jeune B, Vaupel J, McGue M, Christensen K. Apolipoprotein e genotypes: relationship to cognitive functioning, cognitive decline, and survival in nonagenarians. *J Am Geriatr Soc*. 2006;54:654–658. doi:10.1111/j.1532-5415.2005.53554.x
16. Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. *Nat Rev Genet*. 2006;7:436–448. doi:10.1038/nrg1871
17. Deelen J, Beekman M, Uh HW, et al. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. *Aging Cell*. 2011;10:686–698. doi:10.1111/j.1474-9726.2011.00705.x
18. Gerdes LU, Jeune B, Ranberg KA, Nybo H, Vaupel JW. Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: apolipoprotein E gene is a “frailty gene,” not a “longevity gene”. *Genet Epidemiol*. 2000;19:202–210. doi:10.1002/1098-2272(200010)19:3<202::AID-GEPI2>3.0.CO;2-Q
19. Nebel A, Kleindorp R, Caliebe A, et al. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. *Mech Ageing Dev*. 2011;132:324–330. doi:10.1016/j.mad.2011.06.008
20. Sebastiani P, Solovieff N, Dewan AT, et al. Genetic signatures of exceptional longevity in humans. *PLoS One*. 2012;7:e29848. doi:10.1371/journal.pone.0029848
21. Anselmi CV, Malovini A, Roncarati R, et al. Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res*. 2009;12:95–104. doi:10.1089/rej.2008.0827
22. Flachsbart F, Caliebe A, Kleindorp R, et al. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA*. 2009;106:2700–2705. doi:10.1073/pnas.0809594106
23. Li Y, Wang WJ, Cao H, et al. Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. *Hum Mol Genet*. 2009;18:4897–4904. doi:10.1093/hmg/ddp459
24. Soerensen M, Dato S, Christensen K, et al. Replication of an association of variation in the FOXO3A gene with human longevity using both case-control and longitudinal data. *Aging Cell*. 2010;9:1010–1017. doi:10.1111/j.1474-9726.2010.00627.x
25. Willcox BJ, Donlon TA, He Q, et al. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA*. 2008;105:13987–13992. doi:10.1073/pnas.0801030105
26. Murabito JM, Yuan R, Lunetta KL. The search for longevity and healthy aging genes: insights from epidemiological studies and samples of long-lived individuals. *J Gerontol A Biol Sci Med Sci*. 2012;67:470–479. doi:10.1093/gerona/gls089
27. Newman AB, Walter S, Lunetta KL, et al. A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. *J Gerontol A Biol Sci Med Sci*. 2010;65:478–487. doi:10.1093/gerona/glq028
28. Deelen J, Beekman M, Codd V, et al. Leukocyte telomere length associates with prospective mortality independent of immune-related parameters and known genetic markers. *Int J Epidemiol*. 2014.
29. Psaty BM, O'Donnell CJ, Gudnason V, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: design of prospective meta-analyses of genome-wide association studies from 5 cohorts. *Circ Cardiovasc Genet*. 2009;2:73–80. doi:10.1161/CIRCGENETICS.108.829747
30. Deelen J, Beekman M, Uh HW, et al. Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age. *Hum Mol Genet*. 2014. doi:10.1093/hmg/ddu139
31. Yang J, Ferreira T, Morris AP, et al. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet*. 2012;44:369–375, S361–S363.
32. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82. doi:10.1016/j.ajhg.2010.11.011
33. Beekman M, Blanche H, Perola M, et al. Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study. *Aging Cell*. 2013;12:184–193. doi:10.1111/accel.12039
34. Donlon TA, Curb JD, He Q, et al. FOXO3 gene variants and human aging: coding variants may not be key players. *J Gerontol A Biol Sci Med Sci*. 2012;67:1132–1139.
35. Flachsbart F, Moller M, Daumer C, et al. Genetic investigation of FOXO3A requires special attention due to sequence homology with FOXO3B. *Eur J Hum Genet*. 2013;21:240–242.
36. Walter S, Atzmon G, Demerath EW, et al. A genome-wide association study of aging. *Neurobiol Aging*. 2011;32:2109. e2115–e2128. doi:10.1016/j.neurobiolaging.2011.05.026
37. Hong H, Xu L, Liu J, et al. Technical reproducibility of genotyping SNP arrays used in genome-wide association studies. *PLoS One*. 2012;7:e44483. doi:10.1371/journal.pone.0044483
38. Broer L, Lill CM, Schuur M, et al. Distinguishing true from false positives in genomic studies: p values. *Eur J Epidemiol*. 2013;28:131–138. doi:10.1007/s10654-012-9755-x
39. Liu YJ, Zhang L, Pei Y, Papiasani CJ, Deng HW. On genome-wide association studies and their meta-analyses: lessons learned from osteoporosis studies. *J Clin Endocrinol Metab*. 2013;98:E1278–E1282. doi:10.1210/jc.2013-1637
40. Ganna A, Rivadeneira F, Hofman A, et al. Genetic determinants of mortality. Can findings from genome-wide association studies explain variation in human mortality? *Hum Genet*. 2013;132:553–561. doi:10.1007/s00439-013-1267-6
41. Johnson TE. Recent results: biomarkers of aging. *Exp Gerontol*. 2006;41:1243–1246. doi:10.1016/j.exger.2006.09.006
42. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet*. 2013;45:422–427. doi:10.1038/ng.2528
43. Newman AB, Boudreau RM, Naydeck BL, Fried LF, Harris TB. A physiological index of comorbidity: relationship to mortality and disability. *J Gerontol A Biol Sci Med Sci*. 2008;63:603–609.
44. Westendorp RG, van Heemst D, Rozing MP, et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: the Leiden Longevity Study. *J Am Geriatr Soc*. 2009;57:1634–1637. doi:10.1111/j.1532-5415.2009.02381.x
45. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet*. 2010;11:415–425. doi:10.1038/nrg2779
46. Cordell HJ. Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum Mol Genet*. 2002;11:2463–2468.



47. Nelson MR, Kardia SL, Ferrell RE, Sing CF. A combinatorial partitioning method to identify multilocus genotypic partitions that predict quantitative trait variation. *Genome Res.* 2001;11:458–470.
48. Guo X, Meng Y, Yu N, Pan Y. Cloud computing for detecting high-order genome-wide epistatic interaction via dynamic clustering. *BMC Bioinformatics.* 2014;15:102.
49. Gravina S, Vijg J. Epigenetic factors in aging and longevity. *Pflugers Arch.* 2010;459:247–258. doi:10.1007/s00424-009-0730-7
50. Bell JT, Tsai PC, Yang TP, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genet.* 2012;8:e1002629. doi:10.1371/journal.pgen.1002629