THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2022, Vol. 77, No. 8, 1557–1563
https://doi.org/10.1093/gerona/glab378
Advance Access publication December 20, 2021

OXFORD

Special Issue: The FOXO3 Gene and Its Relation to Lifespan and Healthspan

Sex Difference and Interaction of *SIRT1* and *FOXO3* Candidate Longevity Genes on Life Expectancy: A 10-Year Prospective Longitudinal Cohort Study

John S. Ji, ScD,[1,*] Linxin Liu, MMed,[1] Chang Shu, PhD,[2] Lijing L. Yan, PhD,[3] and Yi Zeng, PhD[4,5]

[1]Vanke School of Public Health, Tsinghua University, Beijing, China. [2]Departments of Pediatrics and Systems Biology, Columbia University, New York, New York, USA. [3]Global Health Research Center, Duke Kunshan University, Kunshan, China. [4]Center for Healthy Aging and Development Studies, National School of Development, Peking University, Beijing, China. [5]Center for the Study of Aging and Human Development, Duke Medical School, Durham, North Carolina, USA.

*Address correspondence to: John S. Ji, ScD, Vanke School of Public Health, Tsinghua University, 4th Fl. Mingli Building, Beijing 100084, China. E-mail: johnji@tsinghua.edu.cn

Received: June 29, 2021; Editorial Decision Date: December 5, 2021

Decision Editor: David Le Couteur, MBBS, FRACP, PhD

Abstract

SIRT1 and *FOXO3* are both associated with longevity. Molecular biology research in many organisms (yeast, nematode worm *Caenorhabditis elegans*, and mice mammalian models) shows *SIRT1* acts on the *FOXO* family of forkhead transcription factors to respond to oxidative stress better, shifting processes away from cell death toward stress resistance. Human population studies need epidemiologic evidence. We used an open cohort of 3 166 community-dwelling participants in China with follow-up from 2008 to 2018. The mean age at baseline was 84.6 years. In 16 375 person-years of follow-up, there were 1 968 mortality events. *SIRT1* and *FOXO3* exhibited Mendelian randomization as there was no correlation with each other and with baseline study population characteristics. Some *SIRT1* and *FOXO3* single-nucleotide polymorphisms showed protective effects for mortality risk. The *FOXO3* protective effect was stronger in females, and the *SIRT1* protective effect was stronger in male study participants. We did not see evidence of a synergistic effect of being carriers of both *SIRT1* and *FOXO3* advantageous alleles.

Keywords: Effect modification, FOXO3, Gene–gene interaction, Longevity, Sex difference, SIRT1

Forkhead box "O" 3 (*FOXO3 or FOXO3A*) and Sirtuin 1 (*SIRT1*) are genes associated with longevity. These genes may work together to regulate aging processes, with molecular biology evidence showing *SIRT1* upregulates the FOXO family of Forkhead transcription factors to better respond to cellular stress, induce cell cycle arrest, resistance to oxidative stress, and aid in the insulin signaling pathway (1,2). These experimental findings were conducted in a variety of organisms including in yeast, the nematode worm *Caenorhabditis elegans*, and mice mammalian models (3). FOXO transcription factors and sirtuin deacetylases are critical regulators of mammalian vascular development and disease (4,5). Currently, no studies have assessed the combined effect of *FOXO3* and *SIRT1* on longevity and morbidity in population cohorts. We used a cohort of older Chinese to study the interaction of *SIRT1* single-nucleotide polymorphisms (SNPs) and *FOXO3* SNPs on mortality. First, we assess the individual effect of *FOXO3* and *SIRT1* on mortality. Second, we evaluate whether the effect of *FOXO3* and *SIRT1* varies by sex. Third, we assess the interaction term of *FOXO3* and *SIRT1* to look for synergistic effects.

Method

Study Population

We analyzed the Chinese Longitudinal Healthy Longevity Survey (CLHLS) study. This study collected information from the oldest-old population drawn from rural and urban regions in 23 out of 31 provinces in China. The first survey started in 1998, and there were new participants recruited to replace the deceased older adult during the follow-up surveys in 2000, 2002, 2005, 2008, 2011, 2014, and 2018. We included participants first interviewed in 2008/2009 and

excluded those aged younger than 65, non-Han Chinese, without genetic data, and lost in the first follow-up. The final sample consisted of 3 166 participants.

Genotype Assessment of *FOXO3* and *SIRT1*

The Beijing Genomics Institute performed the genotyping for 13 228 individuals using a customized chip for Chinese ancestry based on the previous CLHLS Genome-Wide Association Study (GWAS). The GWAS genotyping and quality control procedures were reported previously (6). The replication study targeted 27 656 longevity-phenotype-related SNPs. We extracted the same tagging *FOXO3* and *SIRT1* SNPs as previous longevity studies (7,8): *FOXO3* rs4946936, *FOXO3* rs2802292, *FOXO3* rs2253310, *SIRT1* rs12778366, *SIRT1* rs3758391, *SIRT1* rs2273773, and *SIRT1* rs4746720.

We recoded the genotypes following the additive, heterozygote, minor-dominant, and minor-recessive models. The genotype that contains 0, 1, or 2 copies of minor allele was categorized as "AA," "Aa," and "aa." In the additive model, we coded "AA" as 0 (reference group), "Aa" as 1, and "aa" as 2. In the heterozygote model, we coded "Aa" as 0 (reference group), "AA" as 1, and "aa" as 2. In the minor-dominant model, we coded "Aa"/"aa" as 1 and AA as 0. In the recessive model, we coded "aa" as 1 and "Aa"/"AA" as 0. In most analyses, carrying one copy of the minor allele "a" (additive model) is considered to have a decreased risk for mortality.

We further classified the combination of the genotype of *FOXO3* and *SIRT1* into 4 groups: carrying no minor allele of either *FOXO3* or *SIRT1*; carrying at least one minor allele of *FOXO3* and no minor allele of *SIRT1*; carrying no minor allele of *FOXO3* and at least one minor allele of *SIRT1*; and carrying at least one minor allele of both *FOXO3* and *SIRT1*.

Mortality Ascertainment

The next of kin reported the mortality information in the follow-up surveys between 2008 and 2018. The survival time was entered as month counted from the month of the initial interview to the month of death or censoring at the 2018 interview.

Statistical Analysis

We used a Cox proportional hazard model for every candidate SNP to evaluate their effect on mortality individually. We tested the interaction effect by adding the product of one *FOXO3* SNP and one *SIRT1* SNP. For each SNP, we draw the adjusted survival curve based on the expected survival curves calculated based on the Cox model separately for subpopulations (9). We further conducted

Table 1. Population Characteristics at Baseline

	FOXO3 rs2802292 Minor Allele Number			*SIRT1* rs3758391 Minor Allele Number			Overall (*N* = 3 166)
	0 (*N* = 1 590)	1 (*N* = 1 282)	2 (*N* = 294)	0 (*N* = 2 231)	1 (*N* = 855)	2 (*N* = 80)	
Sex: n (%)							
Male	733 (46.1)	602 (47.0)	152 (51.7)	1 041 (46.7)	412 (48.2)	34 (42.5)	1 487 (47.0)
Female	857 (53.9)	680 (53.0)	142 (48.3)	1 190 (53.3)	443 (51.8)	46 (57.5)	1 679 (53.0)
Age (year)							
Mean (*SD*)	84.6 (11.4)	85.6 (11.2)	85.0 (11.0)	84.8 (11.4)	85.8 (10.9)	84.8 (10.9)	85.0 (11.3)
Education year							
Mean (*SD*)	2.11 (3.40)	2.07 (3.37)	2.30 (3.45)	2.13 (3.43)	2.09 (3.33)	1.86 (2.96)	2.11 (3.39)
Missing	4 (0.3)	2 (0.2)	1 (0.3)	6 (0.3)	1 (0.1)	0 (0)	7 (0.2)
Residence: n(%)							
City/town	525 (33.0)	398 (31.0)	98 (33.3)	733 (32.9)	257 (30.1)	31 (38.8)	1 021 (32.2)
Rural	1 065 (67.0)	884 (69.0)	196 (66.7)	1 498 (67.1)	598 (69.9)	49 (61.2)	2 145 (67.8)
Marriage status: n (%)							
Married	617 (38.8)	459 (35.8)	113 (38.4)	838 (37.6)	317 (37.1)	34 (42.5)	1 189 (37.6)
Not married	973 (61.2)	823 (64.2)	181 (61.6)	1 393 (62.4)	538 (62.9)	46 (57.5)	1 977 (62.4)
Exercise: n (%)							
Current	457 (28.7)	358 (27.9)	84 (28.6)	653 (29.3)	229 (26.8)	17 (21.2)	899 (28.4)
Former	107 (6.7)	79 (6.2)	22 (7.5)	148 (6.6)	57 (6.7)	3 (3.8)	208 (6.6)
Never	1 024 (64.4)	845 (65.9)	188 (63.9)	1 429 (64.1)	568 (66.4)	60 (75.0)	2 057 (65.0)
Missing	2 (0.1)	0 (0)	0 (0)	1 (0.0)	1 (0.1)	0 (0)	2 (0.1)
Smoking: n (%)							
Current	345 (21.7)	283 (22.1)	66 (22.4)	501 (22.5)	178 (20.8)	15 (18.8)	694 (21.9)
Former	207 (13.0)	176 (13.7)	34 (11.6)	264 (11.8)	139 (16.3)	14 (17.5)	417 (13.2)
Never	1 037 (65.2)	823 (64.2)	194 (66.0)	1 465 (65.7)	538 (62.9)	51 (63.8)	2 054 (64.9)
Missing	1 (0.1)	0 (0)	0 (0)	1 (0.0)	0 (0)	0 (0)	1 (0.0)
Alcohol drinking: n (%)							
Current	359 (22.6)	277 (21.6)	57 (19.4)	474 (21.2)	198 (23.2)	21 (26.2)	693 (21.9)
Former	176 (11.1)	118 (9.2)	23 (7.8)	216 (9.7)	91 (10.6)	10 (12.5)	317 (10.0)
Never	1 054 (66.3)	887 (69.2)	214 (72.8)	1 540 (69.0)	566 (66.2)	49 (61.2)	2 155 (68.1)
Missing	1 (0.1)	0 (0)	0 (0)	1 (0.0)	0 (0)	0 (0)	1 (0.0)
Body mass index group (kg/m²): n (%)							
<18	487 (30.6)	392 (30.6)	104 (35.4)	689 (30.9)	269 (31.5)	25 (31.2)	983 (31.0)
[18, 25)	924 (58.1)	725 (56.6)	165 (56.1)	1 283 (57.5)	492 (57.5)	39 (48.8)	1 814 (57.3)
[25, 30)	121 (7.6)	111 (8.7)	18 (6.1)	174 (7.8)	65 (7.6)	11 (13.8)	250 (7.9)
≥30	22 (1.4)	27 (2.1)	3 (1.0)	37 (1.7)	13 (1.5)	2 (2.5)	52 (1.6)
Missing	36 (2.3)	27 (2.1)	4 (1.4)	48 (2.2)	16 (1.9)	3 (3.8)	67 (2.1)

stratified analyses by genotype and gender. We created new variables combining the *FOXO3*, *SIRT1*, and gender to compare the single and combined effects intuitively. All models were adjusted for age at baseline and gender. In the sensitivity analyses, we additionally adjusted for education, residence, marriage, exercise, smoking, drinking alcohol, and body mass index (BMI). We calculated hazard ratios (HRs) and 95% confidence intervals (CIs) to indicate associations between SNPs and mortality using R 4.0.0.

Results

We studied a total of 3 166 participants. During 16 375 person-years of follow-up, there were 1 968 mortality events. At baseline, the participants had a mean age of 85 (*SD*: 11), and 53% (n = 1 679) were female (Table 1). The distributions of *FOXO3* and *SIRT1* SNPs were not correlated with each other. We did not see meaningful or consistent trends in the difference of *FOXO3* and *SIRT1* SNP distributions by baseline age, years of education, urban or rural residence location, marital status, exercise frequency, smoking, alcohol consumption, and BMI measurement (Table 1; Supplementary Tables 1 and 2).

Interestingly, the protective effect of *FOXO3* is only evident in females. In Table 2, we can see the protective effect of *FOXO3* homozygous minor allele carriers, but it became more evident when stratified by gender. The relationship was consistent for all *FOXO3* SNPs. Homozygous minor alleles of the 3 *FOXO3* SNPs were associated with lower mortality risk in the additive and recessive model. The protective effect tended to be recessive because there was no significant mortality difference between one minor allele and zero minor alleles. The HR (95% CI) adjusted for age and gender in the recessive model were 0.795 (0.668–0.947) for *rs4946936*, 0.805 (0.689–0.941) for *rs2802292*, and 0.808 (0.692–0.944) for *rs2253310* (Table 2). These associations persisted after adjusting for lifestyle and BMI (data not shown).

For *SIRT1*, the protective effect was seen for some SNPs in the total sample and was only statistically significant for males when stratified by gender (Table 3). Homozygous minor alleles of *rs4746720* were associated with lower mortality risk in the recessive (HR [95% CI]: 0.879 [0.782–0.988]) and the heterozygote model (HR [95% CI]: 0.857 [0.757–0.969]), and the results persisted after additionally adjusting for lifestyle and BMI. However, in the context of multiple comparisons, we cannot infer strong associations given chance findings. There was a borderline negative association between the homozygous minor alleles of *rs3758391* and mortality risk in the heterozygote model (HR [95% CI]: 0.737 [0.538–1.008]). This association became significant after additionally adjusting for lifestyle and BMI (HR [95% CI]: 0.716 [0.517–0.992]). The association tended to only exist in the male, not in the female (Table 3).

We identified significant interactions between *rs4946936* and *rs12778366*, *rs4946936* and *rs3758391*, *rs2802292* and *rs3758391*, and *rs2253310* and *rs2273773* (Table 4). In the stratified analyses, homozygous minor alleles of *rs2802292* had lower mortality risk compared to homozygous major alleles for participants carrying

Table 2. The Association Between *FOXO3* SNPs and Mortality

SNP	Minor Allele Number	Total			Male			Female		
		n	HR (95% CI)	*p*	*n*	HR (95% CI)	*p*	*N*	HR (95% CI)	*p*
rs4946936	0	1 715	Reference	—	788	Reference	—	927	Reference	—
rs4946936	1	1 220	0.931 (0.849–1.022)	.13	585	0.981 (0.854–1.129)	.79	635	0.897 (0.792–1.016)	.087
rs4946936	2	231	0.771 (0.645–0.922)	.004	114	0.912 (0.708–1.175)	.48	117	0.666 (0.517–0.858)	.0016
rs2802292	0	1 590	Reference	—	733	Reference	—	857	Reference	—
rs2802292	1	1 282	0.959 (0.874–1.052)	.38	602	0.96 (0.834–1.105)	.57	680	0.961 (0.849–1.088)	.53
rs2802292	2	294	0.79 (0.672–0.928)	.004	152	0.949 (0.758–1.19)	.65	142	0.667 (0.528–0.843)	.00069
rs2253310	0	1 609	Reference	—	739	Reference	—	870	Reference	—
rs2253310	1	1 261	0.946 (0.862–1.039)	.25	595	0.967 (0.839–1.113)	.64	666	0.933 (0.824–1.057)	.28
rs2253310	2	296	0.789 (0.671–0.926)	.004	153	0.948 (0.758–1.187)	.64	143	0.666 (0.528–0.84)	.00060
rs4946936	0	1 715	Reference	—	788	Reference	—	927	Reference	—
rs4946936	1/2	1 451	0.903 (0.826–0.987)	.025	699	0.969 (0.849–1.107)	.65	752	0.856 (0.76–0.965)	.011
rs2802292	0	1 590	Reference	—	733	Reference	—	857	Reference	—
rs2802292	1/2	1 576	0.924 (0.846–1.01)	.081	754	0.958 (0.838–1.094)	.52	822	0.901 (0.8–1.015)	.087
rs2253310	0	1 609	Reference	—	739	Reference	—	870	Reference	—
rs2253310	1/2	1 557	0.914 (0.836–0.998)	.045	748	0.963 (0.843–1.099)	.57	809	0.879 (0.78–0.99)	.034
rs4946936	0/1	2 935	Reference	—	1 373	Reference	—	1 562	Reference	—
rs4946936	2	231	0.795 (0.668–0.947)	.010	114	0.919 (0.719–1.176)	.50	117	0.698 (0.545–0.894)	.0044
rs2802292	0/1	2 872	Reference	—	1 335	Reference	—	1 537	Reference	—
rs2802292	2	294	0.805 (0.689–0.941)	.0066	152	0.967 (0.779–1.201)	.76	142	0.679 (0.542–0.852)	.00084
rs2253310	0/1	2 870	Reference	—	1 334	Reference	—	1 536	Reference	—
rs2253310	2	296	0.808 (0.692–0.944)	.0073	153	0.963 (0.776–1.194)	.73	143	0.687 (0.548–0.861)	.0011
rs4946936	1	1 220	Reference	—	585	Reference	—	635	Reference	—
rs4946936	0	1 715	1.074 (0.978–1.178)	.13	788	1.019 (0.886–1.172)	.79	927	1.115 (0.984–1.263)	.087
rs4946936	2	231	0.828 (0.69–0.994)	.042	114	0.929 (0.717–1.204)	.58	117	0.743 (0.574–0.961)	.024
rs2802292	1	1 282	Reference	—	602	Reference	—	680	Reference	—
rs2802292	0	1 590	1.043 (0.95–1.145)	.38	733	1.042 (0.905–1.2)	.59	857	1.041 (0.919–1.178)	.53
rs2802292	2	294	0.824 (0.699–0.971)	.021	152	0.989 (0.786–1.244)	.93	142	0.694 (0.548–0.88)	.0025
rs2253310	1	1 261	Reference	—	595	Reference	—	666	Reference	—
rs2253310	0	1 609	1.057 (0.963–1.16)	.25	739	1.035 (0.898–1.191)	.64	870	1.071 (0.946–1.213)	.28
rs2253310	2	296	0.833 (0.707–0.982)	.029	153	0.981 (0.78–1.233)	.87	143	0.714 (0.564–0.904)	.0051

Notes: SNP = single-nucleotide polymorphism; HR = hazard ratio; CI = confidence interval. All models adjusted for baseline age and sex.

homozygous major alleles of *rs3758391* (Supplementary Table 3). Homozygous minor alleles of *rs3758391* had lower mortality risk than homozygous major alleles for participants carrying homozygous major alleles of *rs2802292* (Supplementary Table 4).

There was no significant 3-way interaction of *FOXO3*, *SIRT1*, and gender (Supplementary Table 5).

The protective effect of *FOXO3* and *SIRT1* SNPs was also illustrated in the adjusted survival curve shown in Supplementary Figures 1 and 2. After combining the genotype of *rs2802292* and *rs3758391*, those with at least one minor allele of *rs2802292* and without minor allele of *rs3758391* (blue line), and those with at least one minor allele of *rs3758391* and without minor allele of *rs2802292* (green line) had higher survival rate than those without any minor allele of *rs2802292* and *rs3758391* (red line), and those with at least one minor allele of both *rs2802292* and *rs3758391* (purple line; Supplementary Figure 3).

In Figure 1 and Supplementary Figure 4, *rs2802292* minor allele carriers showed lower mortality risk than *rs2802292* minor allele noncarriers in both male and female *rs3758391* minor allele noncarriers, but this association became reversed in both male and female *rs3758391* minor allele carriers. Meanwhile, the association was stronger in females than males. *rs3758391* minor allele carriers showed lower mortality risk than *rs3758391* minor allele noncarriers in females *rs2802292* minor allele noncarriers, but this association became reversed in both male and female *rs2802292* minor allele carriers. Females had a lower mortality risk than males in all genotype combinations.

Discussion

In this cohort study with up to 10 years of follow-up, we found evidence of a strong protective effect of *FOXO3* against mortality across all the studied SNPs. The effect was only evident in female

Table 3. The Association Between *SIRT1* SNPs and Mortality

SNP	Minor Allele Number	Total			Male			Female		
		n	HR (95% CI)	*p*	*n*	HR (95% CI)	*p*	*n*	HR (95% CI)	*p*
rs12778366	0	2 334	Reference	—	1 090	Reference	—	1 244	Reference	—
rs12778366	1	761	1.046 (0.943–1.161)	.39	363	1.088 (0.934–1.268)	.28	398	1.008 (0.875–1.162)	.91
rs12778366	2	71	1.071 (0.772–1.486)	.68	34	1.202 (0.752–1.921)	.44	37	0.963 (0.609–1.522)	.87
rs3758391	0	2 231	Reference	—	1 041	Reference	—	1 190	Reference	—
rs3758391	1	855	1.054 (0.956–1.162)	.29	412	1.149 (0.993–1.328)	.061	443	0.987 (0.865–1.127)	.85
rs3758391	2	80	0.776 (0.571–1.056)	.11	34	0.591 (0.341–1.025)	.061	46	0.907 (0.626–1.315)	.61
rs2273773	0	1 693	Reference	—	792	Reference	—	901	Reference	—
rs2273773	1	1 246	1.083 (0.987–1.188)	.091	587	1.113 (0.969–1.278)	.13	659	1.06 (0.936–1.201)	.38
rs2273773	2	227	0.986 (0.827–1.175)	.87	108	0.929 (0.709–1.216)	.59	119	1.034 (0.82–1.305)	.78
rs4746720	0	1 039	Reference	—	483	Reference	—	556	Reference	—
rs4746720	1	1 550	1.064 (0.963–1.175)	.22	754	1.012 (0.873–1.173)	.88	796	1.109 (0.97–1.268)	.13
rs4746720	2	577	0.911 (0.799–1.039)	.16	250	0.847 (0.69–1.04)	.11	327	0.959 (0.809–1.138)	.63
rs12778366	0	2 334	Reference	—	1 090	Reference	—	1 244	Reference	—
rs12778366	1/2	832	1.048 (0.947–1.16)	.36	397	1.096 (0.945–1.272)	.23	435	1.005 (0.875–1.154)	.94
rs3758391	0	2 231	Reference	—	1 041	Reference	—	1 190	Reference	—
rs3758391	1/2	935	1.029 (0.935–1.132)	.56	446	1.1 (0.953–1.268)	.19	489	0.98 (0.862–1.114)	.76
rs2273773	0	1 693	Reference	—	792	Reference	—	901	Reference	—
rs2273773	1/2	1 473	1.067 (0.977–1.166)	.15	695	1.082 (0.948–1.236)	.24	778	1.056 (0.938–1.189)	.37
rs4746720	0	1 039	Reference	—	483	Reference	—	556	Reference	—
rs4746720	1/2	2 127	1.019 (0.928–1.12)	.69	1 004	0.969 (0.841–1.115)	.66	1 123	1.062 (0.936–1.204)	.35
rs12778366	0/1	3 095	Reference	—	1 453	Reference	—	1 642	Reference	—
rs12778366	2	71	1.059 (0.764–1.468)	.73	34	1.176 (0.737–1.877)	.50	37	0.961 (0.609–1.516)	.86
rs3758391	0/1	3 086	Reference	—	1 453	Reference	—	1 633	Reference	—
rs3758391	2	80	0.765 (0.563–1.038)	.085	34	0.568 (0.328–0.983)	.043	46	0.911 (0.63–1.318)	.62
rs2273773	0/1	2 939	Reference	—	1 379	Reference	—	1 560	Reference	—
rs2273773	2	227	0.953 (0.803–1.131)	.58	108	0.887 (0.682–1.153)	.37	119	1.009 (0.805–1.266)	.93
rs4746720	0/1	2 589	Reference	—	1 237	Reference	—	1 352	Reference	—
rs4746720	2	577	0.879 (0.782–0.988)	.030	250	0.841 (0.699–1.011)	.066	327	0.904 (0.777–1.051)	.19
rs12778366	1	761	Reference	—	363	Reference	—	398	Reference	—
rs12778366	0	2 334	0.956 (0.861–1.06)	.39	1 090	0.919 (0.788–1.071)	.28	1 244	0.992 (0.861–1.143)	.91
rs12778366	2	71	1.023 (0.732–1.431)	.89	34	1.104 (0.683–1.786)	.69	37	0.955 (0.598–1.526)	.85
rs3758391	1	855	Reference	—	412	Reference	—	443	Reference	—
rs3758391	0	2 231	0.949 (0.86–1.046)	.29	1 041	0.871 (0.753–1.007)	.061	1 190	1.013 (0.887–1.157)	.85
rs3758391	2	80	0.737 (0.538–1.008)	.056	34	0.515 (0.295–0.899)	.020	46	0.919 (0.628–1.345)	.66
rs2273773	1	1 246	Reference	—	587	Reference	—	659	Reference	—
rs2273773	0	1 693	0.923 (0.842–1.013)	.091	792	0.899 (0.783–1.032)	.13	901	0.943 (0.833–1.068)	.36
rs2273773	2	227	0.91 (0.761–1.089)	.30	108	0.835 (0.635–1.097)	.19	119	0.976 (0.769–1.237)	.84
rs4746720	1	1 550	Reference	—	754	Reference	—	796	Reference	—
rs4746720	0	1 039	0.94 (0.851–1.038)	.22	483	0.988 (0.853–1.146)	.88	556	0.902 (0.789–1.031)	.13
rs4746720	2	577	0.857 (0.757–0.969)	.014	250	0.837 (0.69–1.016)	.072	327	0.865 (0.737–1.016)	.078

Notes: SNP = single-nucleotide polymorphism; HR = hazard ratio; CI = confidence interval. All models adjusted for baseline age and sex.

study participants. For *SIRT1*, we found some but not strong protective effects against mortality, with the effect only evident for male study participants. Furthermore, we found an interactive effect of *FOXO3* and *SIRT1*. However, the effect was not synergistic. When *FOXO3* and *SIRT1* genes co-occur, it appears that the *FOXO3* effect is more dominant. Female populations in our study appear to have the most mortality benefit from *FOXO3*.

In 2008, a strong association between *FOXO3* and human longevity was firstly reported by Willcox et al. (10) in a long-lived population of male Americans of Japanese and Okinawan ancestry. This novel finding was then replicated in centenarians of German ancestry (11), followed by other populations, including Caucasian women (12), Italians (13), Chinese (7), and others. Sex difference in genetic determinants of longevity has been suggested to be overlooked in GWAS and observational cohorts (6) because investigators typically do not assume there are gender differences in genetic effects. While women typically live longer than men globally, current studies could not quantify whether this is due to the genetic advantage of women or harmful lifestyle (smoking, drinking) and occupational exposures (job hazard, environmental chemical exposure) of men (14,15). Previous studies suggested that the estrogen level may affect the FOXO3 regulatory region and lead to sex differences in aging (16). However, given the advanced age of our female study participants, which are likely to be postmenopausal, we are not sure if this mechanism holds. In animal models, *FOXO3* phosphorylation was lower in females (17). Phosphorylation represents a reversible mechanism employed by cells to regulate transcription factor activity in response to alterations in the extracellular environment. We lack evidence on whether molecular pathways (transcription factors, transcriptional coregulators) of phosphorylation protein kinases or dephosphorylation by protein phosphatases is different by sex. An earlier study using the same CLHLS cohort found *FOXO3* was

Table 4. Significant Interaction of *SIRT1* and *FOXO* SNPs on Mortality

	Without Interaction		With Interaction		
Minor Allele Number (0 as reference)	HR (95% CI)	*p*	HR (95% CI)	Beta (*SE*)	*p*
rs4946936 one	0.931 (0.849–1.022)	.13	0.917 (0.823–1.021)	−0.087 (0.055)	.11
rs4946936 two	0.771 (0.645–0.922)	.0044	0.739 (0.599–0.912)	−0.303 (0.107)	.0048
rs12778366 one	1.047 (0.944–1.162)	.38	1.022 (0.886–1.178)	0.021 (0.073)	.78
rs12778366 two	1.058 (0.763–1.469)	.73	0.907 (0.588–1.4)	−0.097 (0.221)	.66
rs4946936 one × rs12778366 one	—	—	1.049 (0.844–1.305)	0.048 (0.111)	.66
rs4946936 two × rs12778366 one	—	—	1.087 (0.717–1.648)	0.083 (0.212)	.70
rs4946936 one × rs12778366 two	—	—	1.257 (0.614–2.577)	0.229 (0.366)	.53
rs4946936 two × rs12778366 two	—	—	3.199 (1.076–9.517)	1.163 (0.556)	.037
rs4946936 one	0.929 (0.847–1.02)	.12	0.817 (0.73–0.915)	−0.202 (0.058)	.00047
rs4946936 two	0.769 (0.643–0.919)	.0039	0.748 (0.603–0.927)	−0.291 (0.11)	.0080
rs3758391 one	1.053 (0.955–1.161)	.30	0.894 (0.78–1.025)	−0.112 (0.07)	.11
rs3758391 two	0.767 (0.564–1.043)	.090	0.623 (0.415–0.935)	−0.474 (0.207)	.022
rs4946936 one × rs3758391 one	—	—	1.483 (1.208–1.82)	0.394 (0.105)	.00016
rs4946936 two × rs3758391 one	—	—	1.094 (0.737–1.622)	0.089 (0.201)	.66
rs4946936 one × rs3758391 two	—	—	1.841 (0.968–3.502)	0.61 (0.328)	.063
rs4946936 two × rs3758391 two	—	—	1.014 (0.236–4.362)	0.014 (0.744)	.98
rs2802292 one	0.959 (0.874–1.052)	.38	0.852 (0.761–0.954)	−0.16 (0.058)	.0055
rs2802292 two	0.787 (0.67–0.926)	.0038	0.741 (0.609–0.902)	−0.299 (0.1)	.0028
rs3758391 one	1.054 (0.955–1.162)	.29	0.888 (0.77–1.024)	−0.119 (0.073)	.10
rs3758391 two	0.77 (0.566–1.047)	.095	0.621 (0.398–0.969)	−0.476 (0.227)	.036
rs2802292 one × rs3758391 one	—	—	1.447 (1.177–1.778)	0.369 (0.105)	.00044
rs2802292 two × rs3758391 one	—	—	1.199 (0.841–1.709)	0.182 (0.181)	.31
rs2802292 one × rs3758391 two	—	—	1.576 (0.832–2.984)	0.455 (0.326)	.16
rs2802292 two × rs3758391 two	—	—	1.464 (0.428–5.005)	0.381 (0.627)	.54
rs2253310 one	0.947 (0.863–1.04)	.25	0.832 (0.743–0.932)	−0.184 (0.058)	.0015
rs2253310 two	0.787 (0.67–0.924)	.0035	0.727 (0.597–0.884)	−0.319 (0.1)	.0014
rs3758391 one	1.052 (0.954–1.161)	.31	0.874 (0.759–1.006)	−0.135 (0.072)	.060
rs3758391 two	0.769 (0.566–1.046)	.094	0.605 (0.388–0.944)	−0.502 (0.227)	.027
rs2253310 one × rs3758391 one	—	—	1.5 (1.22–1.845)	0.406 (0.105)	.00012
rs2253310 two × rs3758391 one	—	—	1.269 (0.894–1.801)	0.238 (0.179)	.18
rs2253310 one × rs3758391 two	—	—	1.673 (0.883–3.168)	0.514 (0.326)	.11
rs2253310 two × rs3758391 two	—	—	1.514 (0.443–5.179)	0.415 (0.627)	.51
rs2253310 one	0.943 (0.858–1.035)	.21	1.051 (0.925–1.195)	0.05 (0.065)	.45
rs2253310 two	0.788 (0.671–0.925)	.0036	0.771 (0.613–0.968)	−0.261 (0.116)	.025
rs2273773 one	1.086 (0.99–1.192)	.080	1.178 (1.034–1.342)	0.164 (0.066)	.014
rs2273773 two	0.991 (0.831–1.182)	.92	1.133 (0.887–1.447)	0.125 (0.125)	.32
rs2253310 one × rs2273773 one	—	—	0.81 (0.666–0.984)	−0.211 (0.1)	.034
rs2253310 two × rs2273773 one	—	—	1.049 (0.749–1.469)	0.048 (0.172)	.78
rs2253310 one × rs2273773 two	—	—	0.72 (0.494–1.049)	−0.329 (0.192)	.087
rs2253310 two × rs2273773 two	—	—	0.98 (0.537–1.787)	−0.02 (0.307)	.95

Notes: SNP = single-nucleotide polymorphism; HR = hazard ratio; CI = confidence interval. All models adjusted for baseline age and sex.

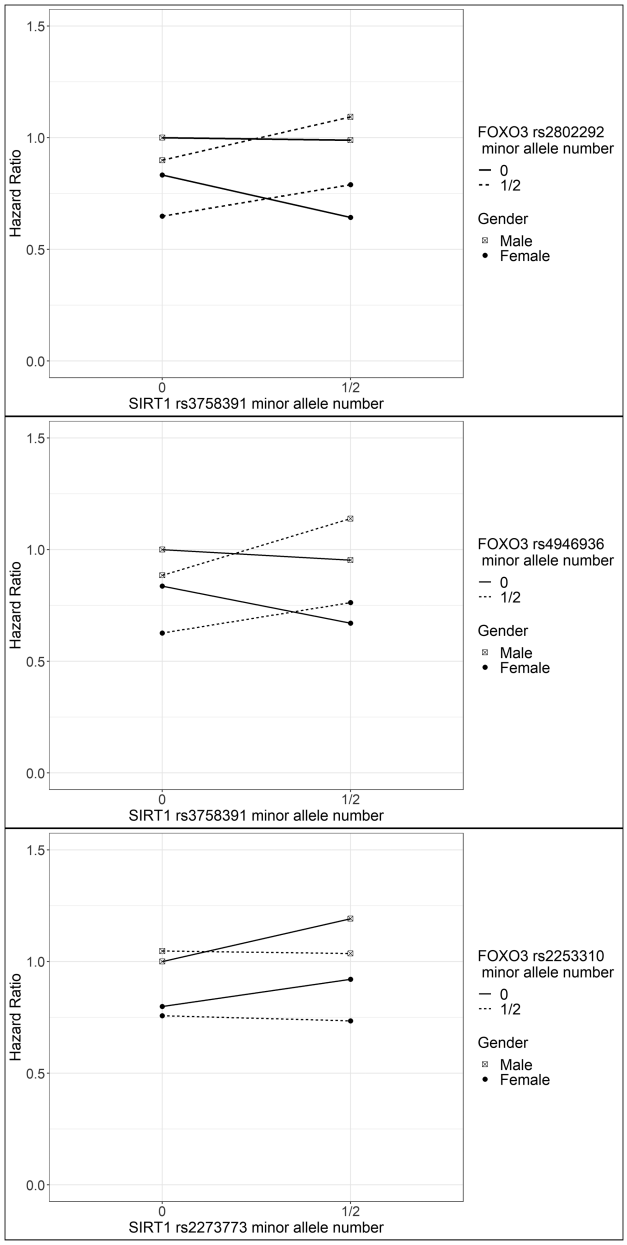

Figure 1. The hazard ratios of different combinations of *FOXO3, SIRT1* SNPs, and gender. *Note:* The model adjusted for age at baseline. SNP = single-nucleotide polymorphism.

associated with expanded life span in both genders (7). However, the study used a case–control study design without the longitudinal follow-up data in our study. Another CLHLS study used polygenic risk score analyses to examine sex differences and found the pathway of sex-specific loci and longevity different between sexes (6). A review article summarizing cohort findings of *FOXO3* on longevity in the cohort of American men of Japanese ancestry along with 11 independent studies of populations of diverse ancestry in multiple different countries did not mention sex differences, possibly due to study design constraints (18). Newer disease-specific studies show that *FOXO3* has been shown to increase the life span of at-risk individuals (men) by protection against cardiometabolic stress (19). Women express higher levels of *FOXO3* mRNA than men in skeletal muscle tissues (20). While our study does not challenge the association of *FOXO3* on longevity in both genders (21), our findings indicate that the contribution may be unequal.

Literature for *SIRT1* and sex differences in the human population also point to the role of estrogen as a modifying factor. A study showed that *SIRT1* protects arteries against menopause-induced senescence and atherosclerosis (22). Another study showed female sex-specific downregulation of SIRT1 in aged hearts (23). Another study points to age and sex modifications of *SIRT1*, with women in their 30s showing the highest *SIRT1* activities (24). The sex differences in the roles of *SIRT1* in many disease outcomes remain to be explored further. No study has presented robust findings on mortality outcomes with respect to gender differences.

As both *FOXO3* and *SIRT1* are recognized longevity genes, prior research assessed how they might work together. In the worm model, NAD+-dependent SIRT1 extends life span by utilizing the *FOXO* transcription factor daf-16. Several findings focusing on mammalian *SIRT1* and *FOXO* have highlighted this genetic interaction. It is hypothesized that mammalian *SIRT1* deacetylates *FOXO* and reduces apoptosis and potentiating FOXO-induced cell cycle arrest. *SIRT1* might increase longevity by shifting *FOXO*-dependent responses away from cell death (19). Other studies point to mechanisms involving bone loss by vitamin D deficiency (25) and may be activated under oxidative stress (26). Likely, the mechanism is multifaceted. The combined and synergistic effect of *FOXO3* and *SIRT1* has not been studied to date in population cohorts. Compared to those only carrying *FOXO3* protective alleles, our study did not see the advantages of carrying both *FOXO3* and *SIRT1* protective alleles with respect to all-cause mortality.

Our study has several notable strengths. First, using an observational study with a high proportion of the oldest old, we can see real-world evidence of the genetic benefit of *FOXO3* and *SIRT1*. This cohort of older individuals also resides in China, which provides generalizability evidence to add to the findings from prior cohorts. Second, our cohort is a longitudinal study with over a decade of follow-up, which means we can capture more mortality events. Third, our cohort is rich in characteristic demographic information, which allows us to conduct stratified analyses and adjust for potential confounders. Limitations of our study findings include the lack of cause-specific mortality information, which would inform which disease outcome *SIRT1* and *FOXO3* may prevent. Our study lacks molecular and epigenetic markers, which does not allow us to see in detail which biological pathway is upregulated or downregulated by genes.

In conclusion, we found strong gene-by-gender interaction. Strong *FOXO3* effects were driven by female study participants, and protective effects of the *SIRT1* effect were in male study participants. There does not appear to be a synergistic effect of being carriers of both longevity candidate gene SNPs. Overall, our findings add novel information that female and male populations may have different benefits from *SIRT1* and *FOXO3* genetic SNPs. Whether this is due to gene–environmental interaction or hormonal differences remains unexplored.

Supplementary Material

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

Funding

The Chinese Longitudinal Healthy Longevity Study (CLHLS) data sets analyzed in this article are jointly supported by the National Key R&D Program

of China (2018YFC2000400), National Natural Sciences Foundation of China (72061137004, 71490732) the U.S. National Institute of Aging of National Institute of Health (P01AG031719), and Duke University School of Medicine and Duke-NUS Medical School/RECA (Pilot)/2019/0051.

Conflict of Interest

None declared.

Acknowledgments

The authors extend appreciation to the participants and investigators of the Chinese Longitudinal Healthy Longevity Survey study.

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