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

The Association Between Longevity-Associated *FOXO3* Allele and Heart Disease in Septuagenarians and Octogenarians: The SONIC Study

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Abstract

The G allele of *FOXO3* gene (single-nucleotide polymorphism; rs2802292) is strongly associated with human longevity. However, knowledge of the effect of *FOXO3* in older populations, men or women, with heart disease is limited. This cross-sectional study in Japan included 1836 older adults in the 70- and 80-year-old groups. DNA samples isolated from buffy coat samples of peripheral blood were used to genotype *FOXO3* (rs2802292). Self-reports were used to obtain heart disease data according to physician diagnosis. Multiple logistic regression was used to test the association by adjusting for the traditional risk factor of heart disease. The prevalence of heart disease in women *FOXO3* G-allele carriers was higher than noncarriers (16.7% vs 11.6%, $p = .022$). The prevalence of coronary heart disease was lower for *FOXO3* G carriers in the 70-year-old group for both sexes (men: 9.3% vs 4.3%, $p = .042$ and women: 10% vs 9%, $p = .079$, respectively). The G allele was negatively associated with heart disease after adjusting for diabetes, hypertension, dyslipidemia, and smoking in men (odds ratio [OR] = 0.70, 95% confidence intervals [CIs], 0.49–0.99, $p = .046$), although the association was weaker after full adjustment. In contrast, women carriers of the *FOXO3* G allele showed a positive association with heart disease after total adjustment (OR = 1.49, 95% CI, 1.00–2.21, $p = .049$). In conclusion, the longevity-associated G allele of *FOXO3* was observed to have contrasting associations with heart disease prevalence according to sex in older Japanese. To further confirm this association, a longitudinal study and a large sample size will be required.

Keywords: Coronary heart disease, G allele of *FOXO3*, Gender difference, Longevity

Heart disease is the leading cause of premature death for the older population worldwide. The condition is a complex disease with multiple determinants, including nongenetic factors and genetic factors. Genetic factors account for about one third of human life-span variation (1). There is abundant evidence to support the genetic factors involved in extreme longevity (2) and their essential role in cardiovascular disease and the life span of older adults (3). Over the last decade, many researchers explored the relationship between Forkhead box class O3 (*FOXO3*) gene variants and human aging. Many studies found that the minor allele (G allele) of *FOXO3* single-nucleotide polymorphism (SNP) rs2802292 strongly correlates with human longevity (4–6), self-rated health (7), and chronic diseases such as coronary heart disease (CHD) in men (8), essential hypertension (HT) (9), and low blood glucose in diabetes patients (10). The G allele of *FOXO3* presence creates an HSF1 binding site, which activates the promoter interaction by chromatin looping, thereby encouraging the expression of *FOXO3* and the activity of the aging hub, while the T allele fails to do so (11,12). Therefore, when *FOXO3* has a good expression, it involves healthy aging and reduces insensitivity to age-related diseases. Thus, the merit of the G allele plays a vital role in human longevity. Several association studies on human longevity highlighted the role of *FOXO3* in protecting against aging through multiple mechanisms such as stress responses, metabolism, immune system, apoptosis, glucose homeostasis, and insulin-like growth factor 1 signaling pathway (13–15). However, there is some uncertainty about the association between G allele of *FOXO3* SNP rs2802292 and longevity in older women (16), and there are few studies in the older populations with heart disease. Therefore, this study aimed to determine the association between G allele of *FOXO3* and heart disease in community-dwelling older populations of men and women.

Method

Study Population

This study was a cross-sectional study of 1836 participants recruited through the SONIC (*S*eptuagenarians, *O*ctogenarians, *N*onagenarians *I*nvestigation with *C*entenarians) study, which is a multidisciplinary research project focused on the health and longevity of older Japanese adults, conducted by dentists, nutritionists, psychologists, sociologists, and geriatric physicians. This is a prospective cohort study of community-dwelling old populations in the eastern and western parts of Japan. The SONIC study was established in 2010 and has a survey every 3 years. It encircles a wide range of 4 age groups (70-, 80-, 90-, and 100-year-old groups) to examine components influencing longevity. The SONIC study includes both the physical and mental health of community-dwelling old populations (17). Participants were older volunteers independently living in private residences and in nursing homes including assisted living participants. They were randomly selected from the local resident registry by mail between 2010 and 2011. The participants were invited to come to the examination venue in groups of 30–50 participants per day. All examinations for each participant were completed in 1 day. The study initially recruited individuals in the 69- to 71- and 79- to 81-year-old groups who registered in 2010 and 2011.

The participants provided written, informed consent onsite before starting the survey. The survey consisted of a medical history interview, blood test, and physical investigation on the same day at a community site. The study was approved by the Institutional Review Board of Osaka University Graduate School of Medicine, Dentistry and Human Sciences, and the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology (Refs. 266, H22-E29, 22 018, and 38).

SNP Selection and Genotyping

DNA was isolated from the buffy coat of peripheral blood using the PureGene system (Gentra Systems, Minneapolis, MN) and quantified using PureGreen staining (Molecular Probes, Eugene, OR). We selected the *FOXO3* longevity-associated allele rs2802292, which has been shown to be strongly correlated with coronary artery disease mortality and longevity in Japanese and other ethnicities (4,5,8,18). The genotype for SNP rs2802292 (G—longevity associated; TT—not associated with longevity) was assessed using the standard TaqMan PCR method and commercial TaqMan Assays. G-allele carriers represent those participants who carry at least one copy of the G allele (TG or GG genotype).

Main Outcome

Heart disease questionnaires asked participants if a physician ever told them that they had a condition of heart disease including angina pectoris, myocardial infarction, atrial fibrillation, heart failure, valvular heart disease, cardiomyopathy, pacemaker, or other forms of cardiac dysfunction that the participants may not have been aware of. CHD was identified as a history of myocardial infarction or angina pectoris.

Covariate Factors and Other Measurements

The covariate factors were based upon the traditional risk factors of cardiovascular disease. HT was defined by systolic blood pressure of at least 140 mmHg and diastolic blood pressure of at least 90 mmHg or being on antihypertensive treatment according to the Japanese Society of Hypertension guideline 2019 (19). Diabetes mellitus (DM) was defined by fasting blood glucose of at least 126 mg/dL or random blood glucose testing of at least 200 mg/dL or HbA1c (National Glycohemiglobin Standardization Program) of at least 6.5% or being on medication for diabetes according to Japanese Clinical Practice Guideline for Diabetes 2016 (20). Dyslipidemia (DLP) was defined by low-density lipoprotein cholesterol of at least 140 mg/dL, high-density lipoprotein cholesterol of less than 40 mg/dL, triglycerides of at least 150 mg/dL, or being on lipid-lowering drugs according to the Japan Atherosclerosis Society (19). The smoking experience was classified based on a questionnaire and then was divided into 2 groups: those who have never smoked and those who have smoked, either currently or in the past.

Other Measurements

Carotid ultrasound was used by the physician to assess the intima–media thickness (IMT), the space between the intima–luminal and medial–adventitial interfaces of the carotid artery. The average IMT value between the right and left carotid arteries was used for analysis.

Statistical Analysis

The statistical analysis was performed using the SPSS 24.0 software. Descriptive statistics analyzed the participant characteristics including the mean ± *SD* and proportions. The *t*-test was used for independent groups, and the comparison of proportions among groups was performed using a chi-squared test. The association between G allele of *FOXO3* and heart disease was assessed using multiple logistic regression to obtain odds ratios (ORs) and

Table 1. The Individual Cardiohealth Characteristics According to *FOXO3* Genotype

	All Participants			Men			Women		
Characteristics	TT	G-Allele Carrier	*p**	TT	G-Allele Carrier	*p**	TT	G-Allele Carrier	*p**
Women, *n* (%)	506 (52.1)	456 (52.7)	.795						
Age group (years)									
70	478 (53.3)	418 (46.7)	.699	226 (52.1)	208 (47.9)	.506	252 (54.5)	210 (45.5)	.245
80	493 (52.4)	447 (47.6)		239 (54.3)	201 (45.7)		254 (50.8)	246 (49.2)	
Hypertension, *n* (%)	724 (75.1)	627 (73.9)	.542	349 (75.5)	305 (76.4)	.758	375 (74.7)	322 (71.6)	.274
Dyslipidemia, *n* (%)	581 (59.8)	531 (61.4)	.183	250 (53.8)	238 (58.2)	.333	331 (65.4)	293 (64.3)	.237
Diabetes, *n* (%)	173 (18.6)	135 (16.2)	.182	94 (21.1)	81 (20.5)	.826	79 (16.3)	54 (12.3)	.084
Smoking ever, *n* (%)	360 (38.8)	329 (40.4)	.500	323 (71.0)	289 (73.5)	.409	37 (7.8)	40 (9.5)	.377
BMI (kg/m^2), mean (*SD*)	22.7 (3.1)	22.7 (2.9)	.848	22.9 (2.8)	22.7 (2.6)	.303	22.5 (3.3)	22.7 (3.2)	.278
HbA1c (mg/dL), mean (*SD*)	5.6 (0.7)	5.5 (0.6)	.340	5.6 (0.7)	5.5 (0.7)	.556	5.6 (0.7)	5.5 (0.6)	.448
LDL-C (mg/dL), mean (*SD*)	118.5 (29.7)	119.6 (28.7)	.675	112.2 (27.4)	114.7 (28.2)	.181	124.3 (30.5)	123.9 (28.5)	.841
HDL-C (mg/dL), mean (*SD*)	60.6 (16.4)	61.0 (16.0)	.436	57.0 (15.2)	58.0 (15.6)	.313	64.0 (16.8)	63.6 (16.0)	.691
SBP, (mmHg), mean (*SD*)	143.2 (18.5)	143.6 (18.8)	.645	143.1 (18.1)	143.8 (18.8)	.569	143.3 (18.9)	143.4 (18.9)	.921
DBP, (mmHg), mean (*SD*)	78.4 (10.5)	78.8 (10.9)	.371.	78.7 (10.4)	79.5 (10.7)	.265	78.1 (10.6)	78.2 (11.1)	.834
Mean IMT (mm), mean (*SD*)	0.82 (0.17)	0.81 (0.16)	.279	0.86 (0.18)	0.83 (0.18)	.025	0.79 (0.16)	0.80 (0.14)	.391
CRP (mg/L), mean (*SD*)	0.15 (0.6)	0.16 (0.5)	.814	0.19 (0.7)	0.19 (0.6)	.968	0.12 (0.4)	0.13 (0.3)	.679
CAD, *n* (%)	66 (53.2)	58 (46.8)	.938	45 (62.5)	27 (37.5)	.099	21 (40.4)	31 (59.6)	.070

Notes: BMI = body mass index; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; SBP = systolic blood pressure; DBP = diastolic blood pressure; mean IMT = mean intima–media thickness; CRP = C-reactive protein; CAD = coronary artery disease. G-allele carrier indicates persons who had at least one G allele of *FOXO3* SNP rs2802292 (TG or GG genotype).

**p* values based on chi-squared test or *t*-test.

95% confidence intervals (CIs). ORs analysis involved 4 models using those variables that were revealed to be traditional risk factors of cardiovascular disease. TT genotype was used for reference and then adjusted for multivariate factors: Model 1 included age group; Model 2 was adjusted for age group and sex; Model 3 was adjusted for HT, DM, DLP, and smoking experience; and Model 4 (fully adjusted) was modified as in Model 2 and included Model 3.

According to the previous studies, there were still differences in sex as a result of genotype, we then investigated whether these correlations were sex-differentiated in models 1–4. The results are described with 95% CI; a 2-sided p value less than .05 was considered significant.

Results

Characteristics for Population

The analyses were performed on data from 1836 participants, including 896 (48.8%) individuals aged 69–71 years and 940 (51.2%) individuals aged 79–81 years. The individual cardiohealth characteristics according to *FOXO3* genotype are presented in Table 1. There were 874 men (47.6%) and 962 women (52.4%). Overall, the prevalence of the G allele was highest in the 80-year-old group. However, when taking sex into account, male G carriers in the 80-year-old group were slightly less prevalent than in the 70-year-old group (47.9% vs 45.7%), while the opposite was true for female G carriers (45.5% vs 49.2%) but no statistically significant differences were found. Male G carriers had lower IMT than noncarriers (p = .025). There was no significant difference in cardiohealth characteristics for women. The top 3 prevailing specific types of heart disease were angina pectoris (5.2%), atrial fibrillation (2.2%), and myocardial infarction (1.7%; Supplementary Figure 1). The prevalence of other forms of heart disease as a function of *FOXO3* genotype is shown in Supplementary Figure 2.

Individual health characteristics according to heart disease are presented in Table 2. The prevalence of heart disease was highest in the 80-year-old group in all participants for both sexes. In considering all participants, the incidence of heart disease was higher in men (p < .001), with HT (p = .002) and ever smoking (p < .001), respectively. Those men who had heart disease were also more likely to have DM (p = .035) and HT (p = .002). Women who had heart disease also had a higher prevalence of DLP (p = .010) and ever smoke experience (p = .043). Multiple adjustments for the association of heart disease are presented in Table 3. The associations with heart disease were observed for age (OR 1.52, 95% CI: 1.16–1.98, p = .002) and sex stratification (men: OR 1.48, 95% CI: 1.04–2.12, p = .031 and women: OR 1.60, 95% CI: 1.05–2.43, p = .027, respectively). HT was also significantly associated with heart disease in all participants (OR 1.55, 95% CI: 1.11–2.17, p = .009) and men (OR 1.97, 95% CI: 1.23–3.18, p = .005).

Considering *FOXO3* genotype, women G carriers were more likely to have heart disease than noncarriers (16.7% vs 11.6%, p = .022; Figure 1). In considering CHD stratified by age and sex, the prevalence of CHD was slightly lower in both men (4.3% vs 9.3%, p = .042) and women (9% vs 10%, p = .079) G carriers than noncarriers in the 70-year-old group (Figure 2), while the 80-year-old group we not found significantly differences. Additionally, in considering sex and age stratification, women G carriers showed a slightly higher prevalence of heart disease than noncarriers in the 70-year-old group (14.1% vs 8.4%, p = .055; Supplementary Figure 3).

The Association Between *FOXO3* Genotype and Heart Disease

The association and OR between G-carrier status and heart disease are presented in Table 3. No association was found in considering all participants, highlighting the importance of taking sex into account. In men, G carriers had a negative correlation with heart disease after DM, HT, DLP, and smoking adjustment (OR 0.70, 95% CI: 0.49–0.99, p = .046). There was a marked contrast in women—G carriers had a slightly positive correlation with heart disease after age adjustment (OR 1.51, 95% CI: 1.04–2.19, p = .030) and after full adjustment (OR 1.49, 95% CI: 1.00–2.21, p = .049). Moreover, we have analyzed stratified by age (70 and 80), but we did not find any significant associations in the results.

Discussion

In this study of community-dwelling older populations, men who carried the G allele of *FOXO3* SNP rs2802292 had a 30% reduction of heart disease risk after adjusting for DM, HT, DLP, and smoking status. In contrast, women who carried the G allele of *FOXO3* had an increased risk of heart disease, by a magnitude of 1.5, after fully adjusting for confounding factors. The association was not found in

Table 2. Individual Health Characteristics According to Heart Disease, Stratified by Sex

	All Participants			Men			Women		
	Heart Disease			Heart Disease			Heart Disease		
Characteristics	No	Yes	p*	No	Yes	p*	No	Yes	p*
Age group (years), *n* (%)									
70	775 (86.5)	121 (13.5)	<.001	363 (83.6)	71 (16.4)	.014	412 (89.2)	50 (10.8)	.012
80	757 (80.5)	183 (19.5)		339 (77.0)	101 (23.0)		418 (83.6)	82 (16..4)	
Sex, *n* (%)									
Men	702 (45.8)	172 (56.6)	<.001						
Women	830 (54.2)	132 (43.4)							
Diabetes, *n* (%)	235 (16.7)	61 (20.6)	.105	122 (19.3)	45 (26.8)	.035	113 (14.5)	16 (12.5)	.544
Hypertension, *n* (%)	1054 (72.9)	247 (81.2)	.002	474 (73.3)	146 (84.9)	.002	580 (72.6)	101 (76.5)	.346
Dyslipidemia, *n* (%)	874 (59.5)	200 (65.8)	.107	365 (55.4)	99 (57.6)	.707	509 (62.9)	101 (76.5)	.010
Smoking, ever, *n* (%)	512 (36.8)	140 (48.6)	<.001	454 (71.2)	124 (74.3)	.429	58 (7.7)	16 (13.2)	.043

*p values based on chi-squared test.

Table 3. Multiple Logistic Regression for the Association Between G-Allele Carriers and Heart Disease

	All Participants		Men		Women	
Risk Factors	OR (95% CI)	p*	OR (95% CI)	p*	OR (95% CI)	p*
Model 1[a]: *FOXO3* G-allele carrier	1.01 (0.79–1.30)	.918	0.74 (0.52–1.04)	.080	1.51 (1.04–2.19)	.030
Model 2[b]: *FOXO3* G-allele carrier	1.02 (0.80–1.31)	.861				
Model 3[c]: *FOXO3* G-allele carrier	0.99 (0.76–1.28)	.924	0.70 (0.49–0.99)	.046	1.52 (1.02–2.25)	.039
Model 4[d]: *FOXO3* G-allele carrier	0.99 (0.76–1.29)	.958	0.70 (0.49–1.00)	.052	1.49 (1.00–2.21)	.049
Women†	0.74 (0.52–1.05)	.094				
Age†	1.52 (1.16–1.98)	.002	1.48 (1.04–2.12)	.031	1.60 (1.05–2.43)	.027
Diabetes†	1.21 (0.87–1.68)	.261	1.47 (0.98–2.21)	.066	0.83 (0.46–1.50)	.531
Hypertension†	1.55 (1.11–2.17)	.009	1.97 (1.23–3.18)	.005	1.23 (0.77–1.98)	.391
Dyslipidemia†	1.00 (1.00–1.00)	.907	1.00 (1.00–1.00)	.860	1.00 (1.00–1.00)	.883
Smoking status, ever†	1.35 (0.95–1.91)	.094	1.18 (0.79–1.77)	.417	1.82 (0.98–3.38)	.058

Notes: 95% CI = 95% confidence interval; OR = odds ratio; [a]Adjusting for age; [b]Adjusting for age and sex; [c]Adjusting for diabetes, hypertension, dyslipidemia, and smoking; [d]For all participants, adjusting for sex, age, diabetes, hypertension, dyslipidemia, and smoking. For sex stratified, adjusting for age, diabetes, hypertension, dyslipidemia, and smoking; *FOXO3* G-allele carrier indicates persons who had at least one G allele of *FOXO3* SNP rs2802292. Shown are homozygotes (GG) and heterozygotes (TG) combined; TT genotype was used as the reference group for calculation of OR.

*p values based on logistic regression adjustment.

†From all adjusted models.

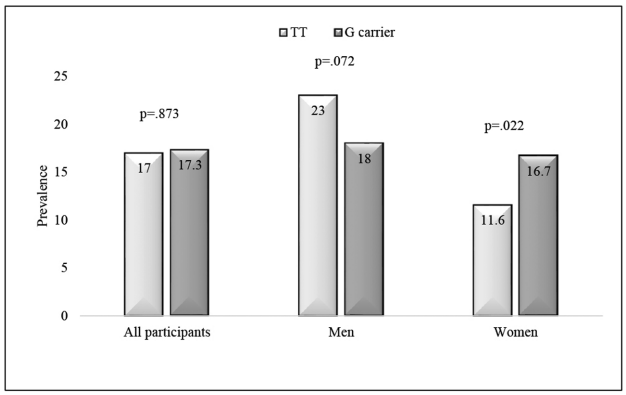

Figure 1. The prevalence of heart disease according to *FOXO3* G-carrier status. G carrier indicates those persons who had at least one G allele for the *FOXO3* SNP rs2802292 (homozygote GG or heterozygote TG), p value was obtained by chi-square analysis.

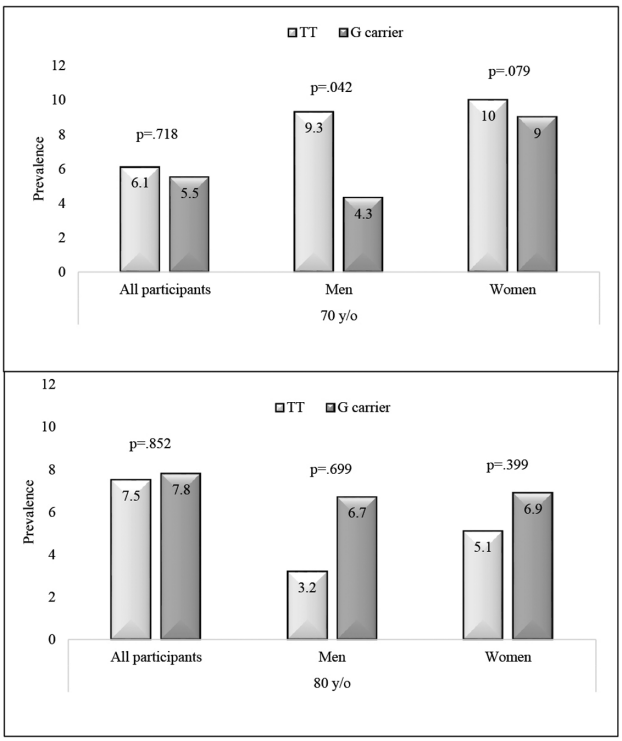

Figure 2. The prevalence of coronary heart disease according to *FOXO3* G-carrier status, stratified by age and sex. G carrier indicates those persons who had at least one G allele for the *FOXO3* SNP rs2802292 (homozygote GG or heterozygote TG), p value was obtained by chi-square analysis.

considering all participants, highlighting potentially differing effects of the *FOXO3* longevity variant on heart disease according to sex.

It is well documented that the G allele of *FOXO3* SNP rs2802292 has a protective effect against all-cause mortality in multiple populations around the world (5,8,18,21,22). It was further shown in a study of aging men, the *FOXO3* G allele had a strong protective effect against CHD mortality (8). The association in women is unclear (16). Our study found that women who carry the *FOXO3* G allele were at higher risk for heart disease than those noncarriers, though the effect on heart disease mortality is uncertain.

In both men and women in the 70-year-old group, the prevalence of CHD was lower in *FOXO3* G-allele carriers though the effect was borderline significant in women, which could be due to the small sample size in this group (Figure 2). Hence, our findings are consistent with the clinical observation reported from the Kuakini Honolulu Heart Program cohort study, where it was shown that CHD prevalence was lower in American men of Japanese ancestry who carry the G allele of *FOXO3* (8). The mechanism for the protective effect of the *FOXO3* G allele of rs2802292 against heart disease in older populations could potentially affect the cardiovascular system in different ways such as autophagy (23), inflammatory function (24), cellular reactive oxygen species production, and apoptosis (25–27). The onset of several diseases is affected by *FOXO3*-dependent regulation of autophagy, such as cardiac hypertrophy (28), diabetic cardiomyopathy (29), and ischemic heart disease (30). Moreover, *FOXO3* is an essential regulator in endothelial cells, where it affects the proliferation of vascular smooth muscle cells and the neointimal hyperplasia

that causes atherosclerosis (31). This in turn likely has significant clinical effects of atherosclerosis, such as coronary artery disease or stroke. Our study found that atherosclerosis (IMT) in those male carriers of the *FOXO3* G allele was lower than noncarriers. In addition, the *FOXO3* longevity variant may have a suppressive effect on the inflammatory cascade (21) which promotes atherosclerosis progression. Furthermore, the *FOXO3* SNP was not found to be associated with the onset of CHD in the 80-year-old group, which could be because aging is the strongest risk factor for the onset of heart disease. Therefore, the positive influence of *FOXO3* SNP is not working on the onset of heart disease in the older population, such as those in the 80-year-old group.

Sex differences in the association between G allele and heart disease in the study could be due to a number of different factors. For example, *FOXO3* likely has clinically relevant effects on multiple aging disorders and chronic diseases, including HT and diabetes (DM). In this study, we found the prevalence of DM in women was significantly lower than in men (data were not shown). Additionally, the issue of selective survival due to cardiovascular mortality in men is higher than in women (32), those women who carry the G allele may live longer and participate more in the study than those who take the G allele.

In conclusion, the G allele of *FOXO3A* SNP rs2802292 was observed to be associated with heart disease but there were sex differences in this association. It was associated with a protective effect against CHD for both sexes. This study's limitations include the relatively small sample size especially for participants with heart diseases, and the medical history of heart disease that was assessed based on self-reports, which could ultimately lead to misclassification bias or recall bias. However, further longitudinal studies are needed to confirm and explore a mechanism of *FOXO3* and should involve a larger sample size.

Supplementary Material

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

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Conflict of Interest

None declared.

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Author Contributions

Conceptualized and designed the study: N.K., Y.A., M.K., Y.G., K.G., H.R., and K.K. Performed data acquisition: all authors. Performed analysis and interpretation: N.K., R.C., R.C.A., D.C.W., and B.J.W. Prepared the manuscript and figures: N.K., R.C.A., M.K., and K.K. All authors approved the final version for submission.

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