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ORIGINAL ARTICLE

Male Aging

# Association between *FOXO3A* gene polymorphisms and human longevity: a meta-analysis

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Numerous studies have shown associations between the *FOXO3A* gene, encoding the forkhead box O3 transcription factor, and human or specifically male longevity. However, the associations of specific *FOXO3A* polymorphisms with longevity remain inconclusive. We performed a meta-analysis of existing studies to clarify these potential associations. A comprehensive search was conducted to identify studies of *FOXO3A* gene polymorphisms and longevity. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by comparing the minor and major alleles. A total of seven articles reporting associations of *FOXO3A* polymorphisms with longevity were identified and included in this meta-analysis. These comprised 11 independent studies with 5241 cases and 5724 controls from different ethnic groups. rs2802292, rs2764264, rs13217795, rs1935949 and rs2802288 polymorphisms were associated with human longevity (OR = 1.36, 95% CI = 1.10–1.69,  $P = 0.005$ ; OR = 1.20, 95% CI = 1.04–1.37,  $P = 0.01$ ; OR = 1.27, 95% CI = 1.10–1.46,  $P = 0.001$ ; OR = 1.14, 95% CI = 1.01–1.27 and OR = 1.24, 95% CI = 1.07–1.43,  $P = 0.003$ , respectively). Analysis stratified by gender indicated significant associations between rs2802292, rs2764264 and rs13217795 and male longevity (OR = 1.54, 95% CI = 1.33–1.79,  $P < 0.001$ ; OR = 1.38, 95% CI = 1.15–1.66,  $P = 0.001$ ; and OR = 1.39, 95% CI = 1.15–1.67,  $P = 0.001$ ), but rs2802292, rs2764264 and rs1935949 were not linked to female longevity. Moreover, our study showed no association between rs2153960, rs7762395 or rs13220810 polymorphisms and longevity. In conclusion, this meta-analysis indicates a significant association of five *FOXO3A* gene polymorphisms with longevity, with the effects of rs2802292 and rs2764264 being male-specific. Further investigations are required to confirm these findings.

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**Keywords:** *FOXO3A*; longevity; meta-analysis; polymorphism

## INTRODUCTION

Human life expectancy is a complex phenotype affected by various environmental and genetic factors. Genetic factors contribute to human aging and longevity through the modulation of biologic pathways,<sup>1</sup> and genetic variation in the insulin/"insulin-like" growth factor-1 signaling pathway has been shown to affect human longevity.<sup>2,3</sup> Recent studies found associations between variants of the *FOXO3A* gene (also known as *FKHR-L1*), which forms part of the insulin/insulin-like growth factor-1 signaling pathway, and human longevity.<sup>4–7</sup>

The human *FOXO3A* gene (6q21, MIM 602681) encodes the multifunctional forkhead box O3 transcription factor, which belongs to the FOXO (Forkhead box, class O) subfamily of transcription factors characterized by an evolutionarily conserved DNA-binding domain. In mammals, the FOXO subfamily consists of four genes, *FOXO1*, *FOXO3*, *FOXO4* and *FOXO6*. A previous study suggested that *FOXO1*, *FOXO4* and *FOXO6* were not associated with human longevity.<sup>8</sup> FOXO proteins are involved in diverse cellular and physiological processes, including cell proliferation, apoptosis, reactive oxygen species response, longevity, cancer and regulation of the cell cycle and metabolism.<sup>9</sup>

In 2008, Willcox *et al.*<sup>4</sup> first reported a strong association between the *FOXO3A* gene and longevity in long-lived American men of Japanese ancestry. Anselmi *et al.*<sup>5</sup> subsequently validated this association in men from a southern Italian centenarian study. These findings suggest that *FOXO3A* may play a role in determining longevity, especially in males. Several case-control studies have investigated the associations of *FOXO3A* polymorphisms with longevity, and *FOXO3A* has been linked to human longevity in Japanese,<sup>4</sup> Italian,<sup>5</sup> German<sup>6</sup> and Chinese<sup>7</sup> populations. However, these studies reported conflicting results regarding the roles of some specific polymorphisms in determining longevity.

No comprehensive meta-analysis has yet been conducted to evaluate the associations between specific *FOXO3A* polymorphisms and longevity. We therefore performed a meta-analysis of 11 case-control studies to explore the associations of eight *FOXO3A* polymorphisms (rs2802292, rs2764264, rs13217795, rs1935949, rs2802288, rs2153960, rs7762395 and rs13220810) with longevity, and to clarify any gender-specific effects of these polymorphisms. The results of this study will provide the most comprehensive evidence for the roles of specific *FOXO3A* gene polymorphisms in determining human longevity.

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## MATERIALS AND METHODS

### Study selection

We performed a systematic search of literature published prior to July 2013 in PubMed, EMBASE, the Cochrane Library, ScienceDirect, Springerlink, Scirus, Chinese Biomedical (CBM, Chinese) and Chinese National Knowledge Infrastructure (CNKI, Chinese) databases using the keywords 'FOXO3A', 'FKHR-L1', 'FOXO3', 'longevity', 'long life', 'long lifespan' and combined phrases. Additional literature was collected by cross-referencing original and review articles. No language restrictions were applied. A flow diagram of the search strategy is shown in **Figure 1**. Studies were required to meet the following inclusion criteria: (i) published before July 2013; (ii) case-control or cohort studies; (iii) case subjects were long-lived, control subjects had average lifespans or were younger people; results from different ethnicities or different genders within a study were treated as separate studies and (iv) enough information to calculate odds ratios (ORs) with 95% confidence intervals (CIs). Exclusion criteria were: (i) not case-control or cohort studies evaluating the association of the FOXO3A polymorphisms with longevity; (ii) case reports, letters, reviews, editorials or correspondence articles; (iii) studies based on incomplete raw data and (iv) studies that contained duplicate data.

### Data extraction

All data were extracted independently by two investigators (JB and XS) according to prespecified selection criteria. Discrepancies were resolved by a third investigator (YH). The following data were extracted from the included studies: name of the first author, publication year, ethnicity of the population, available genotype, numbers of cases and controls, ages of the cases and controls and results of studies.

### Statistical analysis

Meta-analyses were performed for polymorphisms investigated in at least two studies. The strength of the associations between FOXO3A gene polymorphisms and longevity were estimated by ORs and 95% CIs. ORs and respective 95% CIs were calculated by comparing the minor and major alleles.

Heterogeneity among studies in terms of degree of association was assessed using  $\chi^2$  tests. The  $I^2$  statistic was used to estimate the percentage of variation between the results that was caused by

heterogeneity, rather than sampling error ( $I^2 < 50\%$  was treated as no significant heterogeneity). When heterogeneity was detected, the OR was pooled according to a random-effect model using the DerSimonian and Laird method; otherwise, a fixed-effect model using the Mantel-Haenszel method was selected.<sup>10</sup> The significance of the pooled OR was determined by the Z-test.

Sensitivity analyses were carried out to evaluate the stability of the results of the meta-analysis. The influence of individual studies was evaluated by estimating the pooled ORs in the absence of each study.<sup>11</sup>

Potential publication bias was assessed by Begg's adjusted correlation test<sup>12</sup> and visual inspection of Begg's funnel plots. For all analyses, a  $P$  value  $< 0.05$  was considered to represent statistical significance for all comparisons. All statistical analyses were performed using Stata statistical software, version 12.0 (Stata Corp., College Station, TX, USA).

## RESULTS

### Study selection and characteristics

We initially identified 3543 results relevant to the search terms in the selected databases (**Figure 1**). After reading the titles and abstracts, 11 articles<sup>2,4-7,13-18</sup> were included for full-text review. Of these, two articles<sup>2,16</sup> were excluded because they were reviews and one article<sup>17</sup> was removed because it was a haplotype analysis. After further screening, one further article<sup>18</sup> was excluded because it was a biodemographic analysis. A total of seven articles<sup>4-7,13-15</sup> including 11 independent case-control studies that examined the associations of FOXO3A gene polymorphisms with longevity were finally selected for meta-analysis. Six of the articles were written in English and one article<sup>15</sup> was in Chinese. Of the 11 studies, seven focused on both genders, two on males and two on females. The characteristics of the studies are presented in **Table 1**.

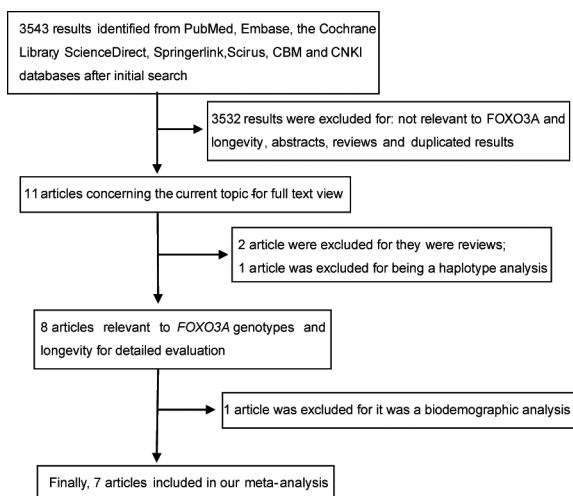
### rs2802292 polymorphism

#### Analysis in overall population

The association of the FOXO3A rs2802292 polymorphism with longevity was investigated in five independent studies including a total of 2521 cases and 2537 controls. The distributions of genotype and allele frequencies in each case-control study are shown in **Table 2**. The genotype distributions in the control groups were consistent with the Hardy-Weinberg equilibrium. The Q-test of heterogeneity was significant and the analysis was therefore conducted using a random-effect model. There was a significant association between rs2802292 polymorphism and prolonged life span in the overall population, when comparing the G and T alleles (Forest plot shown in **Figure 2**), TG + TT vs GG, TG + GG vs TT, GG vs TT and TG vs TT genotypes (OR = 1.36, 95% CI = 1.10–1.69,  $P = 0.005$ ; OR = 0.612, 95% CI = 0.395–0.949,  $P = 0.028$ ; OR = 1.507, 95% CI = 1.025–2.216,  $P = 0.037$ ; OR = 1.97, 95% CI = 1.11–3.51,  $P = 0.021$  and OR = 1.38, 95% CI = 0.98–1.96,  $P = 0.066$ , respectively). Furthermore, we included three studies<sup>4,5,7</sup> in which the controls were highly consistent with the Hardy-Weinberg equilibrium to validate the association and the results again showed a strong association of the rs2802292 polymorphism with longevity (OR = 1.47, 95% CI = 1.31–1.65,  $P < 0.001$ ).

#### Analysis in males

Two independent studies<sup>4,5</sup> reported a strong association between FOXO3A genotype and extreme male longevity. We therefore conducted a sub-analysis of four studies (990 cases and 1650



**Figure 1:** Flow diagram of the study selection process. CBM: Chinese Biomedical; CNKI: Chinese National Knowledge Infrastructure.

**Table 1: Characteristics of studies investigating associations of FOXO3A polymorphisms with longevity**

Reference	Ethnic group	Polymorphisms	Sample size		Age (year)	
			Case M F	Control M F	C Case	Control
Willcox et al. <sup>4</sup> (2008)	Japanese	rs2764264, rs13217795, rs2802292	213 213 0	402 402 0	>95	<81
Anselmi et al. <sup>5</sup> (2009)	Italians	rs9486902, rs2802288, rs10499051, rs2802292, rs13220810, rs2764264, rs7341233, rs17598747, rs9285397, rs12202209, rs9480866, rs12207868, rs13217795, rs2153960, rs380029, rs1935949	281 281 0	195 195 0	90-108	18-48
Anselmi et al. <sup>5</sup> (2009)	Italians	rs9486902, rs2802288, rs10499051, rs13220810, rs2764264, rs7341233, rs17598747, rs9285397, rs12202209, rs9480866, rs12207868, rs2153960, rs380029, rs1935949	199 0 199	140 0 140	90-109	18-48
Flachsbart et al. <sup>6</sup> (2009)	German	rs2274776, rs1571631, rs6911407, rs768023, rs2802288, rs2883881, rs12200646, rs2802290, rs13220810, rs7762395, rs9400239, rs3800231, rs1268170, rs473268, rs479744, rs519007	1031 NA NA	731 NA NA	95-110	60-75
Flachsbart et al. <sup>6</sup> (2009)	French	rs768023, rs7762395, rs3800231	535 NA NA	553 NA NA	≥100	18-70
Li et al. <sup>7</sup> (2009)	Chinese	rs2253310, rs2802292, rs4946936	761 183 578	1056 682 374	≥100	Younger individuals
Pawlikowska et al. <sup>13</sup> (SOF) (2009)	Caucasian	rs1044891, rs1935949, rs2153960, rs3778588, rs4946935, rs9486902, rs9486913	293 0 293	603 0 603	≥92	≤79
Pawlikowska et al. <sup>13</sup> (CHS) (2009)	Caucasian	rs1044891, rs1935949, rs2153960, rs3778588, rs4946935, rs9486902, rs9486913	279 131 148	797 419 378	≥92	≤79
Pawlikowska et al. <sup>13</sup> (AJC) (2009)	Caucasian	rs1044891, rs1935949, rs2153960, rs3778588, rs4946935, rs9486902, rs9486913	383 97 286	363 155 208	95-108	43-94
Soerensen et al. <sup>14</sup> (2010)	Danish	rs9486902, rs12206094, rs2802292, rs13220810, rs2764264, rs7762395, rs13217795, rs9400239, rs9398172, rs3800231, rs479744	1089 313 776	736 371 365	Oldest old	Middle-aged
Li et al. <sup>15</sup> (2010)	Chinese	rs2764264, rs2802288, rs2802290, rs2802292, rs7762395, rs9400239, rs13217795, 13220810	177 47 130	148 58 90	90-110	48-89

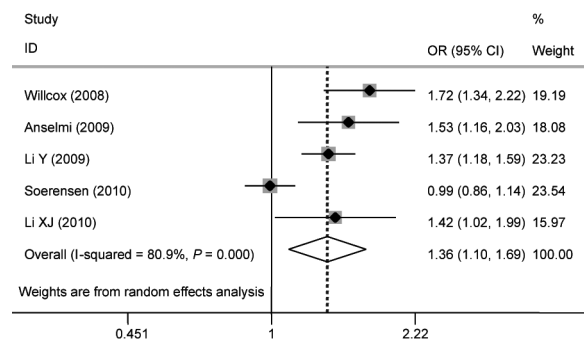
F: female; M: male; NA: not available; AJC: Ashkenazi Jewish Centenarians; CHS: Cardiovascular Health Study; SOF: Study of Osteoporotic Fractures

**Table 2: rs2802292 genotype distributions and allele frequencies in cases and controls**

Author (year)	Genotype, n (%)		Allele frequency, n (%)		HWE for controls P-value
	Cases	Controls	Cases	Controls	
	GT	GT	T	T	
	TT	TT			
Willcox et al. <sup>4</sup> (2008)	26 (12) 106 (50) 81 (38)	26 (6) 153 (38) 223 (55)	158 (37.1) 268 (62.9)	205 (25.5) 599 (74.5)	0.999
Anselmi et al. <sup>5</sup> (2009)	62 (25.92) 121 (50.63) 56 (23.43)	24 (13.95) 92 (53.49) 56 (32.56)	245 (51.3) 233 (48.7)	140 (40.7) 204 (59.3)	0.90
Soerensen et al. <sup>14</sup> (2010)	141 (12.96) 544 (49.91) 404 (37.13)	83 (11.32) 357 (48.43) 296 (40.25)	826 (37.91) 1352 (62.09)	523 (35.54) 849 (64.46)	0.28
Li et al. <sup>7</sup> (2009)	NA	NA	460 (30.2) 1062 (69.8)	507 (24) 1605 (76)	0.74
Li et al. <sup>15</sup> (2010)	NA	NA	125 (35.3) 229 (64.7)	82 (27.7) 214 (72.3)	0.50

HWE: hardy-weinberg equilibrium; N: number; NA: not available

controls) using a random-effect model to investigate the role of the rs2802292 polymorphism, specifically in male longevity. The



**Figure 2: Meta-analysis of the association between rs2802292 and longevity (G vs T). CI: confidence interval; OR: odds ratio.**

rs2802292 polymorphism showed a strong association with longevity in males (OR = 1.54, 95% CI = 1.33–1.79, P < 0.001).

**Analysis in females**

Stratified analysis of the rs2802292 polymorphism in females included two studies (1354 cases and 739 controls) and was conducted using a random-effect model. There was no significant association of the rs2802292 polymorphism with female longevity (OR = 1.16, 95% CI = 0.86–1.57, P = 0.34). The Forest plot is shown in **Figure 3**.



**rs2764264 polymorphism****Analysis in overall population**

The association between the FOXO3A rs2764264 polymorphism and longevity was investigated in five independent studies including a total of 1959 cases and 1621 controls. There was no significant between-study heterogeneity by Q-test and the analysis was therefore conducted using a fixed-effect model. We detected a significant association of rs2764264 with longevity in terms of the C vs the T allele (OR = 1.20, 95% CI = 1.04–1.37,  $P = 0.01$ ).

**Analysis in males**

Two studies including 807 cases and 968 controls were included in a subgroup analysis of rs2764264 in males. The Q-test of heterogeneity was not significant and the analysis was conducted using a fixed-effect model. A significant association between rs2764264 and male longevity was observed (OR = 1.38, 95% CI = 1.15–1.66,  $P = 0.001$ ).

**Analysis in females**

Two studies (975 cases and 505 controls) were included in a stratified analysis to explore the relationship between rs2764264 and female-specific longevity. The result showed absence of association of the rs2764264 polymorphism with female longevity (OR = 0.93, 95% CI = 0.76–1.14,  $P = 0.508$ ).

**rs13217795 polymorphism****Analysis in overall population**

Four independent studies with a total of 1706 cases and 1481 controls were included in the meta-analysis of rs13217795. The Q-test of heterogeneity was not significant and the analysis was conducted using a fixed-effect model. There was a significant association between the FOXO3A rs13217795 polymorphism and longevity in terms of the C vs the T allele (OR = 1.27, 95% CI = 1.10–1.46,  $P = 0.001$ ).

**Analysis in males**

We only conducted a male-specific meta-analysis in three studies, because of insufficient data. The Q-test of heterogeneity was not significant and the data were analyzed using a fixed-effect model. The rs13217795 polymorphism showed a significant association with male longevity (OR = 1.39, 95% CI = 1.15–1.67,  $P = 0.001$ ).

**rs1935949 polymorphism****Analysis in overall population**

A meta-analysis of the association of the FOXO3A rs1935949 polymorphism with longevity was conducted in five independent

studies, including 1435 cases and 2098 controls. The Q-test of heterogeneity was not significant and a fixed-effect model was therefore used to conduct the analysis. We found a significant association between the rs1935949 polymorphism and longevity in terms of the C allele vs the T allele (OR = 1.14, 95% CI = 1.01–1.27).

**Analysis in females**

Two studies (492 cases and 743 controls) were included in the subgroup analysis to investigate any female-specific effect of the rs1935949 polymorphism on longevity. There was significant between-study heterogeneity by Q-test and the analysis was therefore conducted using a random-effect model. No significant association between this polymorphism and female longevity was found (OR = 1.03, 95% CI = 0.83–1.23).

**rs2802288 polymorphism**

Four independent studies comprising 1688 cases and 1214 controls were included in the meta-analysis of the FOXO3A rs2802288 polymorphism. There was no significant between-study heterogeneity by Q-test and the analysis was conducted using a fixed-effect model. A significant association of the rs2802288 polymorphism with prolonged life span was detected in terms of the A allele versus the G allele (OR = 1.24, 95% CI = 1.07–1.43,  $P = 0.003$ ).

**rs2153960 polymorphism****Analysis in overall population**

A meta-analysis of the FOXO3A rs2153960 polymorphism was performed based on five independent studies (1435 cases and 2098 controls). The Q-test of heterogeneity was not significant and the analysis was conducted using a fixed-effect model. The result showed no significant association between this polymorphism and longevity in terms of the C allele versus the T allele (OR = 1.11, 95% CI = 0.98–1.23).

**Analysis in females**

Although rs2153960 was not associated with longevity in the overall population, we investigated its effect in females by analysis of data from two suitable studies. There was no association of rs2153960 with female longevity (OR = 1.07, 95% CI = 0.87–1.28).

**rs7762395 polymorphism**

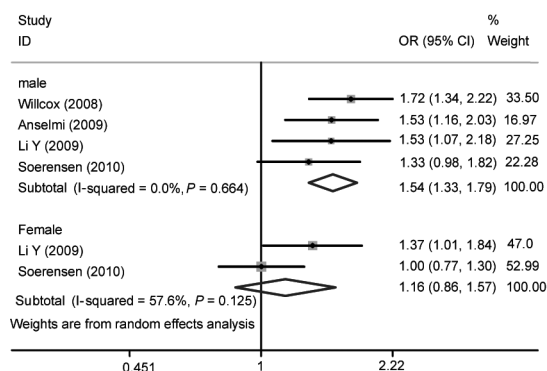
The association of the FOXO3A rs7762395 polymorphism with longevity was investigated in four independent studies including 2832 cases and 2168 controls. The Q-test of heterogeneity was not significant and the analysis was conducted using a fixed-effect model. The result showed absence of association between the rs7762395 polymorphism and prolonged life span in terms of the A allele versus the G allele (OR = 1.11, 95% CI = 0.95–1.30,  $P = 0.173$ ).

**rs13220810 polymorphism****Analysis in overall population**

A meta-analysis of the association between the FOXO3A rs13220810 polymorphism and longevity included four independent studies with a total of 1746 cases and 1219 controls. The Q-test of heterogeneity was not significant and a fixed-effect model was used. There was no significant association of the rs13220810 polymorphism with longevity when comparing the C allele versus the T allele (OR = 0.90, 95% CI = 0.76–1.06,  $P = 0.217$ ).

**Analysis in males**

Two studies (594 cases and 566 controls) were subjected to subgroup analysis for male-specific effects. There was no significant



**Figure 3:** Meta-analysis of the association between rs2802292 and longevity (G vs T): subgroup analysis by gender. CI: confidence interval; OR: odds ratio.

between-study heterogeneity by Q-test and a fixed-effect model was therefore used. No significant association between rs13220810 and male longevity was observed (OR = 0.77, 95% CI = 0.61–1.01,  $P = 0.33$ ).

#### Analysis in females

We conducted a stratified analysis in females including two available studies (975 cases and 505 controls) and using a fixed-effect model, but no significant association of the rs13220810 polymorphism with female longevity was detected (OR = 0.97, 95% CI = 0.76–1.25,  $P = 0.838$ ). Summary of the results of this meta-analysis is listed in **Table 3**.

#### Sensitivity analysis

Sensitivity analyses were performed after the sequential removal of each eligible study to assess the influence of each individual study on the pooled OR. Sensitivity analyses revealed that one independent study by Soerensen *et al.*<sup>14</sup> was the main source of heterogeneity in the analysis of the rs2802292 polymorphism; the heterogeneity was effectively reduced when this study was excluded (G vs T: OR = 1.46, 95% CI = 1.31–1.63,  $I^2 = 0$ ,  $P_{\text{heterogeneity}} = 0.481$ ). In addition, no single study changed the pooled ORs qualitatively, suggesting that the results were relatively stable.

#### Publication bias

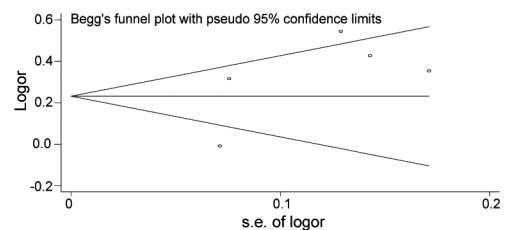
We conducted Begg's test to evaluate potential publication bias. There was no statistical evidence of publication bias regarding analysis of the rs2802292 polymorphism (G vs T:  $P = 0.806$ ) and funnel plots suggested no substantial asymmetry (**Figure 4**). There was no publication bias for other results.

## DISCUSSION

Aging is the result of complex interactions among genetic, epigenetic and environmental factors, but genetic components appear to have a strong impact on survival to extreme age.<sup>19</sup> Human longevity is

considered to be a multifactorial phenotype with a genetic contribution of 20%–30%.<sup>20,21</sup> Genome-wide association studies using single nucleotide polymorphism (SNP) analysis have successfully identified loci for various quantitative traits.<sup>22</sup> Since *APOE* was confirmed as the first longevity gene,<sup>23</sup> several genes have been considered as candidate longevity genes. Increasing numbers of studies have revealed associations between polymorphic variants of the *FOXO3A* gene and longevity.<sup>16,17</sup> *FOXO3A* polymorphisms have repeatedly been identified in numerous populations with the ability to survive into old age, but the findings remain inconsistent, indicating the need to investigate these polymorphisms at a meta-analytical level.

The current study, to the best of our knowledge, represents the first meta-analysis of the associations between the rs2802292, rs2764264, rs13217795, rs2802288, rs7762395 and rs13220810 polymorphisms and human longevity and the most comprehensive meta-analysis to evaluate the association of *FOXO3A* polymorphisms with longevity. The pooled ORs indicated that rs2802292, rs2764264, rs13217795, rs1935949 and rs2802288 were linked to longevity, while subgroup analyses validated significant associations of rs2802292 and rs2764264 polymorphisms with longevity in males, but not females.



**Figure 4:** Begg's funnel plot for publication bias in selection of studies regarding the rs2802292 polymorphism (G vs T). s.e.: standard error.

**Table 3: Meta-analysis of associations between FOXO3A gene polymorphisms and longevity**

Polymorphisms	Comparisons	Sample size		No. of studies	Test of association				Test of heterogeneity		
		Case	control		OR	(95% CI)	P	Model	$\chi^2$	P value	$I^2$
rs2802292	G vs T	2521	2537	5	1.36	1.10-1.69	0.005	R	20.9	0.00	80.9
	TG+TT vs GG	1583	1333	3	0.61	0.39-0.94	0.028	R	5.64	0.06	64.5
	TG+GG vs TT	1583	1333	3	1.50	1.02-2.21	0.037	R	9.00	0.011	77.8
	GG vs TT	1583	1333	3	1.97	1.11-3.51	0.021	R	8.11	0.017	75.3
	TG vs TT	1583	1333	3	1.38	0.98-1.96	0.066	R	6.64	0.036	69.9
Male	G vs T	990	1650	4	1.54	1.33-1.79	0.00	R	1.58	0.664	0.0
Female	G vs T	1354	739	2	1.16	0.86-1.57	0.34	R	2.36	0.125	57.6
rs2764264	C vs T	1959	1621	5	1.20	1.04-1.37	0.01	F	7.03	0.135	43.1
	C vs T	807	968	3	1.38	1.15-1.66	0.001	F	1.00	0.608	0.0
rs13217795	C vs T	975	505	2	0.93	0.76-1.14	0.508	F	0.00	0.953	0.0
	C vs T	1706	1481	4	1.27	1.10-1.46	0.001	F	5.07	0.167	40.8
rs1935949	C vs T	807	968	3	1.39	1.15-1.67	0.001	F	0.82	0.664	0.0
	C vs T	1435	2098	5	1.14	1.01-1.27	NA	F	7.65	0.105	47.7
Female	C vs T	492	743	2	1.03	0.83-1.23	NA	R	4.66	0.031	78.5
rs2802288	A vs G	1688	1214	4	1.24	1.07-1.43	0.003	F	4.83	0.185	37.9
rs2153960	C vs T	1435	2098	5	1.11	0.98-1.23	NA	F	6.9	0.141	42.0
	C vs T	492	743	2	1.07	0.87-1.28	NA	R	5.17	0.023	80.7
rs7762395	A vs G	2832	2168	4	1.11	0.95-1.30	0.173	F	0.51	0.917	0.0
rs13220810	C vs T	1746	1219	4	0.90	0.76-1.06	0.217	F	3.63	0.305	17.3
	C vs T	594	566	2	0.77	0.61-1.01	0.33	F	0.04	0.838	0.0
	C vs T	975	505	2	0.97	0.76-1.25	0.838	F	0.78	0.378	0.0

CI: confidence interval; NA: not available; P value not available (meta-analysis conducted using the effect model, so no P value); OR: odds ratio

Although the *FOXO3A* gene has been considered as a convincing longevity gene and some SNPs in *FOXO3A* have been associated with human longevity, the mechanisms responsible for this effect remain unclear. Common polymorphisms with a modest effect on lifespan have been identified in the *APOE* gene.<sup>24</sup> Interestingly, although the *FOXO3A* rs2802292, rs2764264, rs13217795, rs1935949 and rs2802288 polymorphisms were associated with human longevity, the rs2153960, rs7762395 and rs13220810 polymorphisms showed no such association, indicating that different *FOXO3A* polymorphisms could have potentially large effects on aging phenotypes and longevity. Small differences in *FOXO3A* could theoretically modify several downstream genes related to DNA binding, protein-protein interactions, cell cycle progression, apoptosis and metabolism.<sup>4</sup>

Our study suggests that the rs2802292 polymorphism is strongly associated with human longevity. The most familiar *FOXO3A* intronic SNP rs2802292 is characterized by a G to T transversion. A twin cohort study suggested that carriers of the minor G allele of rs2802292 showed reduced fasting plasma insulin and lower incremental area under the curve 0–120 min for insulin following oral glucose loading compared with non-carriers, indicating that the minor G allele was associated with enhanced peripheral and hepatic insulin sensitivity, possibly mediated through increased *FOXO3A* mRNA expression.<sup>25</sup> Improved insulin sensitivity has been considered as a feature of long-lived individuals. However, a previous study found no associations between *FOXO3A* SNPs (rs2802288, rs1935949, rs9372190, rs2883881, rs2764264, rs13217795, rs9480867 and rs3800227) and type 2 diabetes and related parameters, suggesting that these SNPs influence longevity via an entirely different mechanism.<sup>26</sup> These findings indicate that different *FOXO3A* polymorphisms may affect human longevity through different mechanisms.

The rs2764264, rs13217795, rs1935949 and rs2802288 polymorphisms are all intronic SNPs and are thought to be associated with human longevity. However, these polymorphisms have largely been explored in case-control studies and other investigations to uncover their functions and mechanisms are rare. A previous study suggested that coding variants of the *FOXO3A* gene may not be key players in human aging.<sup>27</sup> Coding genes account for less than one-third of the evolutionarily conserved genome.<sup>28</sup> It is possible that variations do not need to affect coding genes and intronic SNPs of the *FOXO3A* gene may play important roles in longevity.

A novel finding of the present study was the significant association between rs2802292 and rs2764264 polymorphisms and male longevity (OR = 1.54, 95% CI = 1.33–1.79,  $P < 0.001$  and OR = 1.38, 95% CI = 1.15–1.66,  $P = 0.001$ , respectively). Neither of these polymorphisms showed any association with female longevity, suggesting that rs2802292 and rs2764264 manifest male-specific influences on longevity. rs13217795 was also associated with male longevity, while rs1935949 was not linked to female longevity; however, we were unable to conduct stratified analyses of these two polymorphisms in both genders because of insufficient data, and the existence of gender-specific influences on longevity thus remains unclear. The genetic contribution to longevity may be affected by gender; gender is a major variable in the genetics of longevity, and men and women follow different strategies to reach longevity.<sup>29</sup> Several genes involved in determining life span have been found to have different influences on the probability of achieving longevity in men and women,<sup>30</sup> and some investigations have already reported a gender gap in the impact of genetic factors on longevity.<sup>30–37</sup> Specifically, three previous studies observed similar male-specific differences between young people and centenarians.<sup>31–33</sup> The profound effect of the insulin/

insulin-like growth factor-1 signaling pathway on the reproductive/hormonal system, which differs between males and females, suggests that variants that affect genes in this pathway become gender-specific enriched/depleted as the populations age.<sup>5</sup> Our study suggests that men carrying the minor G allele of the rs2802292 polymorphism or the minor C allele of the rs2764264 polymorphism live longer than non-carriers. These male-specific longevity polymorphisms may serve as markers to help identify potentially long-lived men.

Heterogeneity is an important issue in meta-analysis. We detected significant between-study heterogeneity in the analysis of the rs2802292 polymorphism. However, sensitivity analyses identified one independent study<sup>14</sup> as the main source of the heterogeneity, which was effectively decreased when this study was excluded. This study<sup>14</sup> was conducted in a Danish population, suggesting that certain effects of genetic variants may be ethnic-specific. In addition to ethnicity, environmental factors, such as socioeconomic factors, medical security and living environment, may influence the genetic associations and thus contribute to heterogeneity in these studies. No single study exhibited excessive influence, suggesting that the results of this meta-analysis were relatively stable. In addition, Begg's funnel plots and Begg's tests did not detect any publication bias, indicating that the results were unbiased.

This study had several limitations that should be taken into account when considering its contributions. First, all the case-control studies included in our meta-analysis were observational studies. Second, heterogeneity among studies existed in some comparisons of polymorphisms. Third, the language selection bias should be considered. Our analysis was based on English and Chinese electronic databases, even though there was no language restriction in the initial search strategy. Fourth, the limited sample size and insufficient data meant that stratified analysis by gender could only be performed for some polymorphisms and subgroup analysis by ethnicity or age group was also restricted. Finally, because genotypes and allele frequency distributions were unavailable for some studies, we only evaluated allelic associations for most of the polymorphisms, and analyses of other comparative genetic models were restricted in some polymorphisms, except for rs2802292. These factors should be taken into consideration when interpreting the results of this meta-analysis.

In summary, this meta-analysis based on 5241 cases indicated that five *FOXO3A* gene polymorphisms are associated with longevity. rs2802292 and rs2764264 are male-specific longevity polymorphisms that could act as markers to identify potentially long-lived men. Larger, well-designed studies, as well as Genome-wide association studies, are needed to confirm these findings. Moreover, future studies should evaluate gene-gene and gene-environment interactions to clarify the role of the *FOXO3A* gene in longevity.

#### AUTHOR CONTRIBUTIONS

SCZ and QC conceived and designed the study. JMB and XLS performed the literature searches and extracted the data. JMB, XLS and SCZ performed the statistical analysis and wrote the manuscript. YQH, HLZ, CL, TZ and WC were involved in the interpretation of the results. All authors read and approved the final manuscript.

#### COMPETING INTERESTS

The authors declare no competing interests.

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