MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 2966-2975
DOI: 10.12659/MSM.913788

Received: 2018.10.25
Accepted: 2019.01.02
Published: 2019.04.22

Longevity-Associated Forkhead Box O3 (*FOXO3*) Single Nucleotide Polymorphisms are Associated with Type 2 Diabetes Mellitus in Chinese Elderly Women

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDE Yu-Qin Mao
BCD Jin-Feng Liu
EFG Bing Han
AEFG Li-Shun Wang

Institute of Fudan-Minhang Academic Health System, Minhang Hospital, Fudan Univesity, Shanghai, P.R. China

Corresponding Author: Li-Shun Wang, e-mail: lishunwang@fudan.edu.cn
Source of support: This work is supported by the National Natural Science Foundation of China (81472758) and Shanghai Sailing Program (Grant No. 17YF1416700)

Background: This study aimed to investigate the association of single nucleotide polymorphisms (SNPs) of Forkhead box O3 (*FOXO3*) gene with type 2 diabetes mellitus (T2D).

Material/Methods: A total of 843 elderly residents from east China were enrolled in this study, which included 426 patients with type 2 diabetes and 417 controls. Four SNPs were analyzed by qPCR. Genotype frequencies of the 4 SNPs in *FOXO3* of the patients and controls were analyzed using logistic regression analysis. The association between each SNP and clinical indicators was analyzed by linear regression analysis.

Results: None of the 4 *FOXO3* variants, rs13217795, rs2764264, rs2802292, and rs13220810, were associated with the risk of type 2 diabetes compared to controls. However, rs13217795, rs2764264, and rs2802292 were associated with lower blood glucose levels. Notably, further subgroup analysis indicated that the longevity-associated alleles of *FOXO3* SNP (rs13217795, rs2764264, and rs2802292) were associated with lower blood glucose levels in women (TC versus TT, –0.724 mmol/L, *P*=0.005; CC versus TT, –1.093 mmol/L, *P*=0.03; TC versus TT, –0.801 mmol/L, *P*=0.002; CC versus TT, –1.212 mmol/L, *P*=0.001; TG versus TT, –0.754 mmol/L, *P*=0.004; and GG versus TT, –1.150 mmol/L, *P*=0.001) but not in men.

Conclusions: The results indicated that longevity-associated *FOXO3* variants were correlated with lower blood glucose levels in elderly women with type 2 diabetes in east China.

MeSH Keywords: Diabetes Mellitus, Type 2 • Forkhead Transcription Factors • Polymorphism, Single Nucleotide

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/913788

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Type 2 diabetes (T2D), a chronic metabolic disorder with impaired insulin secretion and reduced insulin sensitivity, has become one of the major diseases that impair modern human longevity, and has been increasingly prevalent around the world in the past decades, especially in China [1]. In 1980s, less than 1% of Chinese people suffered from diabetes. However, the recent national survey showed that the prevalence of diabetes has reached 11.6% [2]. The progression of T2D is attributed to environmental and genetic factors as well as their interaction, and it is important to explore and reveal the genetic basis involved in T2D [3–5]. Diabetes and increased blood glucose levels are tightly linked to premature death and shortened lifespan expectancy [6,7]. It has been reported that children of long-lived parents had a lower risk of diabetes [8]. Current research on aging-related disorders usually have a small sample size of elderly people involved. The management of hyperglycemia should be individualized, so reassessing the risk factors of hyperglycemia in the elderly is necessary [9].

The classical ageing insulin/IGF-1 signaling (IIS) pathway plays a key part in metabolism and the pathogenesis of T2D, and its dysfunction directly results in glucose homeostasis disorder [10–12]. Forkhead box O3 gene (*FOXO3*), a forkhead family member located at chromosome 6q21, encodes a transcription factor with the typical domain of this family, a conserved DNA-binding domain, which is a key regulator of insulin/IGF1 signaling (IIS) cascade and has been reported to influence aging and longevity in different organisms [13–18]. Its binding sites have been revealed in the promoters of its key genes such as G6Pase and PEPCK [19]. *FOXO3* is expressed in adipocytes, muscle cells, and liver cells, which are major insulin sensitive tissues and also in insulin producing pancreatic beta cells [19–21]. As a research hotspot, *FOXO3* single nucleotide polymorphisms have been proven to be associated with human longevity in different populations in the past decade [22–27]. A total of 6499 (5372 intron) single-nucleotide variants in *FOXO3* have been found, 135 of which are common variants (minor allele frequency greater than or equal to 0.05). *FOXO3* common variants are associated with healthy aging and longevity [23,28,29]. Given that *FOXO3* SNPs and T2D influence human longevity, some studies have investigated whether there is a genetic basis for *FOXO3* SNPs of T2D patients. In a study of Indian populations, no association between *FOXO3* common variants and diabetes was found [30]. Sun et al. revealed that several *FOXO3* variants were associated with lower fasting hyperglycemia [9]. Intriguingly, glucose tolerance tests in *FOXO3* knockout mice exhibited a declined rate of glucose uptake after overnight fasting [31].

In our study, a meta-analysis was carried out, and it was found that *FOXO3* longevity-associated SNPs rs13217795, rs2764264, and rs2802292 were tightly associated with longevity. The roles of *FOXO3* variants in diabetes (n=426) and controls (n=417) were also investigated by comparing the distribution and clinical biochemical parameters in an elderly population from east China.

Material and Methods

SNP selection

Of the 12 *FOXO3* SNPs (rs2764264, rs2802292, rs12206094, rs7762395, rs9400239, rs479744, rs1935949, rs4946935, rs13217795, rs2802288, rs2153960, and rs13220810) reported to be associated with longevity, 9 SNPs (rs2764264, rs2802292, rs7762395, rs9400239, rs479744, rs13217795, rs2802288, rs2153960, and rs13220810) were included in this study and 3 SNPs (rs12206094, rs1935949, and rs4946935) were not included as only 1 study was available. The top 3 longevity-associated *FOXO3* variants polymorphism (rs13217795, rs2764264, and rs2802292) and rs13220810 as a control were selected [18,22–26,29,32–36].

SNP genotyping

Genomic DNA was isolated from blood samples by using the TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). The SNPs were genotyped by TaqMan probes (Applied Biosystems, Darmstadt, Germany) and quantitative real-time polymerase chain reaction (qRT-PCR) was conducted by using a Quantstudio 7 Flex system. We used 10 ng of genomic DNA for each 5 μL reaction with 2.5 μL TaqMan universal PCR MasterMix (Applied Biosystems, Darmstadt, Germany). VIC and FAM were used for labeling the forward and reverse primers (designed by Applied Biosystems), respectively. The amplification process was as follows: 60°C for 1 minute, followed by 95°C for 10 minutes and 95°C for 15 seconds, and finally 60°C for 1 minute. A total of 45 cycles were run and the completed PCRs were then read by the Allelic Discrimination Sequence Detector Software (Applied Biosystems).

Study participants

A total of 843 elderly residents from east China were enrolled in this study. There were 426 T2D patients and 417 control participants. The protocol was approved by the ethics committee of Minhang Hospital, and informed consent was signed by all participants. Blood samples of participants were harvested for DNA extraction. All the T2D patients were defined according to the 1999 World Health Organization (WHO) criteria, which suggested a fasting plasma glucose (FPG) concentration ≥7.0 mmol/L (126 mg/dL). The controls met the following criteria: FPG <7 mmol/L, no family history of T2D, no oral hypoglycemic agents and glycosylated hemoglobin (HbAlc) <6.5%.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Statistical analysis

Meta-analysis was performed on the *FOXO3* SNPs that were investigated in at least 2 studies. The strength of the associations between *FOXO3* gene SNPs and longevity was indicated by odds ratios (ORs) and 95% confidence intervals (95% CIs) based on the allelic comparison. Data were analyzed using Stata12.1, and forest plot was employed.

SPSS version 22.0 (SPSS, Chicago, IL, USA) was employed for statistical analysis. Student's independent *t*-test was used for the comparison of the variables between the cases and controls. The Hardy-Weinberg equilibrium in the T2D and control groups was analyzed by a chi square goodness of fit test. Logistic regression analysis was used to analyze the distribution of genotype frequencies in cases and controls. Logistic regression analysis was used to calculate the OR and 95% CI of the association between genotype and patients/control.

Results

Meta-analysis of the association between FOXO3 variants and longevity

Many SNPs have been revealed in *FOXO3* and associations between 12 specific SNPs of *FOXO3* and longevity have been demonstrated. We used a meta-analysis to quantitatively synthesize any longevity effects of 9 SNPs based on the allelic comparison of C versus T, G versus T, A versus G, or C versus A. We initially identified 14 601 results relevant to the search terms in the selected databases. After reading the titles and abstracts, 13 articles were included for full-text review. As shown in Figures 1–3 and Table 1, the top 3 longevity-associated *FOXO3* variants were rs2802292 (OR: 1.28, 95% CI: 1.15–1.44), rs13217795 (OR: 1.23, 95% CI: 1.16–1.31), and rs2764264 (OR: 1.20, 95% CI: 1.12–1.28). There was no relationship between rs13220810 (OR: 0.97, 95% CI: 0.87–1.09) and longevity.

Clinical and biochemical parameters of the participants

To test the relationship between T2D and longevity-associated *FOXO3* variants, 426 T2D patients and 417 controls were recruited. Table 2 shows that weight, body mass index (BMI), waist circumferences, hip circumference, triglyceride (TG), fasting glucose (GLU), and glycosylated hemoglobin (HbAlc) were significantly higher in the T2D group (all $P < 0.05$), while high-density lipoprotein (HDL) was lower ($P<0.005$) compared to the control group. Moreover, sex, height, total cholesterol (TC), low-density lipoprotein (LDL), as well as hemoglobin (HgB) remained unchanged compared to the control group.

Association of FOXO3 variants and the morbidity of T2D

As shown in Table 3, the 3 *loci* in *FOXO3* we selected were in Hardy-Weinberg Equilibrium status ($P>0.05$) in the controls. The genotype distributions of the 3 longevity-related *FOXO3* SNP rs13217795, rs2764264, and rs2802292, with rs13220810 as control, are shown in Table 4. In the χ^2 analysis of these 4 *FOXO3* SNPs, there was no significant correlation between *FOXO3* SNPs and T2D in both men and women ($P>0.05$). The frequency of minor allele homozygotes in diabetic versus euglycemic patients was 7.09% versus 6.52%, 6.38% versus 6.80%, 5.19% versus 6.73%, and 4.72% versus 5.60% for rs2802292, rs2764264, rs13217795, and rs13220810 respectively.

The risk of T2D in individuals of different FOXO3 genotypes

To further verify the aforementioned findings, the risk of diabetes in individuals of different *FOXO3* variants was analyzed. As shown in Table 5, the risk of diabetes was slightly changed in individuals of different genotypes, but the difference was not significant ($P>0.05$), except for rs13217795 ($P=0.037$). The genotype of *FOXO3* was not associated with T2D as evidenced by logistic regression analysis.

Association of FOXO3 genotype and glucose in diabetes patients

Whether *FOXO3* SNPs were correlated with diabetes-related clinical chemistry values in all participants was further analyzed. The clinical data of the participants and their genotypes related to diabetes are shown in Table 6. Intriguingly, the longevity-associated alleles of *FOXO3* were associated with lower blood glucose levels when compared to wild type alleles for homozygous/heterozygous rs13217795 (8.29±1.03/8.55±1.71 versus 8.96±1.96), rs2764264 (8.35±1.13/8.54±1.64 versus 8.99±2.0), and rs2802292 (8.15±0.95/8.57±1.64 versus 8.98±2.03) in diabetes patients. In contrast, there was no significant difference in blood glucose levels for rs13220810 (8.86±2.15/8.81±2.00 versus 8.72±1.75) variations in T2D patients.

FOXO3 variants and blood glucose in different gender groups

Furthermore, the T2D patients were divided into male and female groups. It was found that the longevity-associated allele of *FOXO3* SNPs (rs13217795, rs2764264, and rs2802292) were associated with lower blood glucose in female patients (TC versus TT, –0.724 mmol/L, $P=0.005$, CC versus TT, –1.093 mmol/L, $P=0.03$; TC versus TT, –0.801 mmol/L, $P=0.002$, CC versus TT, –1.212 mmol/L, $P=0.001$ and TG versus TT, –0.754 mmol/L, $P=0.004$, GG versus TT, –1.150 mmol/L, $P=0.001$) (Table 7), but not in male patients (Table 7).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

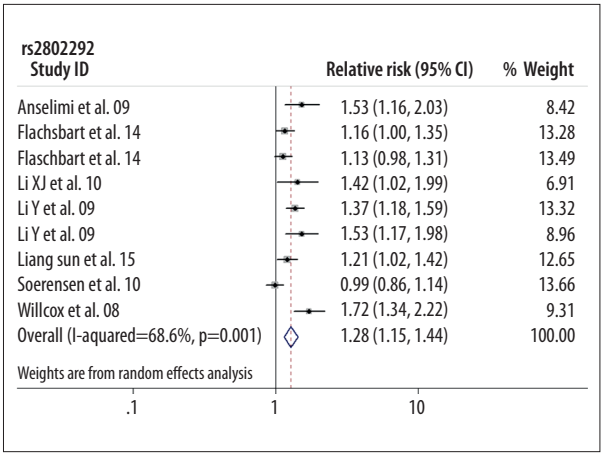

Figure 1. Meta-analysis of the association between rs2802292 and longevity. CI – confidence interval; OR – odds ratio.

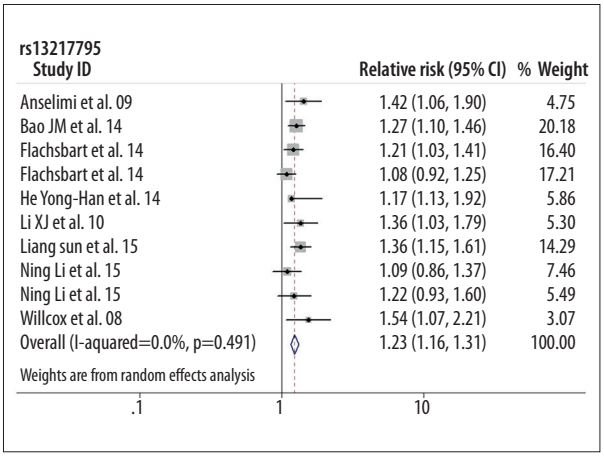

Figure 2. Meta-analysis of the association between rs13217795 and longevity. CI – confidence interval; OR – odds ratio.

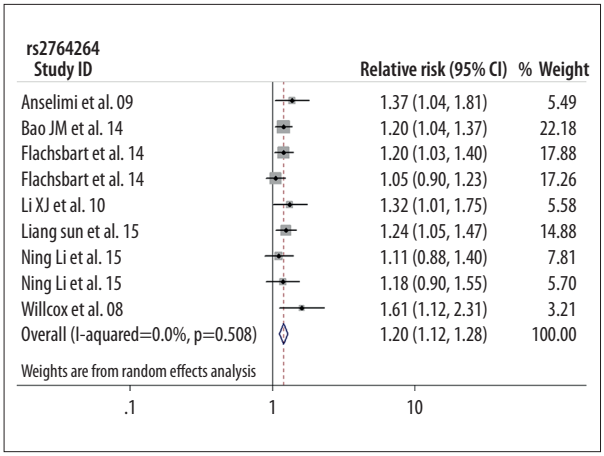

Figure 3. Meta-analysis of the association between rs2764264 and longevity. CI – confidence interval; OR – odds ratio.

Discussion

Few studies had focused on T2D of elderly patients, and age is an important risk factor of T2D. There are 135 common, non-coding SNPs in human *FOXO3* and many of these SNPs have been consistently associated with longevity in global populations [27,29]. In this study, the 3 longevity-associated variants rs13217795, rs2764264, and rs2802292 of *FOXO3* in elderly individuals were investigated. Intriguingly, our results found for the first time that the *FOXO3* longevity-associated variants of rs13217795, rs2764264, and rs2802292 were associated with low blood glucose levels in Chinese elderly women with diabetes ($P<0.05$).

Previous research showed that rs2802292, rs2764264, and rs13217795 were associated with a series of disorders, including

Table 1. The summarized ORs and 95% CIs of each SNP.

SNP	OR	LCI	UCI	Number of study
rs2802292	1.24	1.15	1.33	13
rs13217795	1.23	1.16	1.31	10
rs264264	1.20	1.12	1.28	9
rs2802288	1.19	1.07	1.32	6
rs9400239	1.18	1.09	1.29	4
rs479744	1.17	1.01	1.36	3
rs7762395	1.15	1.04	1.27	5
rs2153960	1.05	0.83	1.33	3
rs13220810	0.97	0.87	1.09	8

SNP – single nucleotide polymorphism; OR – odds ratio; LCI – lower confidence interval; UCI – upper confidence interval.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Clinical and biochemical parameters of the study participants.

Variable	Diabetes (n=426)	Non-diabetes (n=417)	*P*
Sex (Male: Female)	213: 213	197: 220	0.423
Age (years)	67.73±6.61	66.80±6.54	**0.039**
Height (m)	1.61±0.08	1.62±0.08	**0.102**
Weight (kg)	65.55±9.41	62.88±9.73	**<0.001**
BMI (kg/m^2)	25.40±2.95	24.04±2.87	**<0.001**
Waist circumferences (cm)	86.84±7.99	83.41±8.09	**<0.001**
Hip circumference (cm)	95.08±5.66	93.34±5.72	**<0.001**
HDL (mmol/L)	1.20±0.28	1.25±0.28	**0.005**
LDL (mmol/L)	3.02±0.87	3.01±0.67	**0.881**
TG (mmol/L)	1.94±1.41	1.68±1.02	**0.003**
TC (mmol/L)	5.17±1.05	5.16±0.78	**0.954**
SBP (mmHg)	148.81±22.36	140.64±19.23	**<0.001**
DBP (mmHg)	82.96±11.84	81.10±10.17	**0.0028**
GLU (mmol/L)	8.75±1.83	5.94±0.37	**<0.001**
HgB (g/L)	143.98±17.34	142.92±12.79	**0.314**
HbAlc (%)	7.64±1.23	6.33±0.94	**<0.001**

N – number; BMI – body mass index; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglyceride; TC – total cholesterol; SBP – systolic pressure; DBP – diastolic pressure; GLU – glucose; HgB – hemoglobin; HbAlc – glycosylated hemoglobin. Except the data for number, data are expressed as mean ± SE. Unpaired *t*-test and ANOVA were used to compare the difference between groups. Note that *P* was the result from the total participate, Significant *P* values are shown in bold.

Table 3. The Hardy-Weinberg equilibrium in diabetes and controls.

SNPs	N11	N12	N22	N1	N2	*P*
rs2764264						
All participants	448	332	55	1228	442	0.59
Case	218	178	27	614	232	0.27
Control	230	154	28	614	210	0.8
rs13217795						
All participant	453	336	50	1242	436	0.28
Case	216	185	22	617	229	**0.036**
Control	237	151	28	625	207	0.6
rs2802292						
All participant	444	336	57	1224	450	0.6
Case	215	178	30	608	238	0.47
Control	229	158	27	616	212	1

HWE – Hardy-Weinberg equilibrium; N11 – the number of major allele homozygote; N12 – the number of heterozygote; N22 – the number of minor allele homozygote; N1 – the number of major allele (2*N11+N12); N2 – the number of minor allele (2*N22+N12). *P* are calculated using Fisher's exact test, significant *P* values are shown in bold.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 4. Contingency table analysis of FOXO3 genotype frequencies in patients with type 2 diabetes mellitus compared with controls.

SNPs	ALL					Females					Males				
rs13217795	**TT**	**TC**	**CC**	**χ^2**	**P**	**TT**	**TC**	**CC**	**χ^2**	**P**	**TT**	**TC**	**CC**	**χ^2**	***P***
Diabetes	216	186	22	5.253	0.072	103	100	10	3.910	0.142	113	85	12	3.731	0.155
Controls	237	151	28			113	88	19			124	63	9		
rs2764264	**TT**	**TC**	**CC**	**χ^2**	**P**	**TT**	**TC**	**CC**	**χ^2**	**P**	**TT**	**TC**	**CC**	**χ^2**	***P***
Diabetes	218	178	27	1.93	0.381	106	95	11	1.809	0.405	112	83	16	3.563	0.168
Controls	230	154	28			109	90	18			121	64	10		
rs2802292	**TT**	**TG**	**GG**	**χ^2**	**P**	**TT**	**TG**	**GG**	**χ^2**	**P**	**TT**	**TG**	**GG**	**χ^2**	***P***
Diabetes	215	178	30	1.693	0.429	102	95	15	0.195	0.907	113	83	15	2.255	0.324
Controls	229	158	27			109	93	15			120	65	12		
rs13220810	**TT**	**TC**	**CC**	**χ^2**	**P**	**TT**	**TC**	**CC**	**χ^2**	**P**	**TT**	**TC**	**CC**	**χ^2**	***P***
Diabetes	301	103	20	0.771	0.680	156	46	10	2.345	0.310	145	57	10	0.262	0.877
Controls	281	107	23			145	59	13			136	48	10		

P-value for genotype in additive model. SNPs – single-nucleotide polymorphisms.

Table 5. Effects of genotype on type 2 diabetes risk.

SNPs		ALL				Females				Males			
		OR	**95%CI**	***P***		**OR**	**95%CI**	***P***		**OR**	**95%CI**	***P***
rs13217795	TT	1*			TT	1			TT	1		
	TC	1.352	1.018–1.794	**0.037**	TC	1.247	0.843–1.844	0.275	TC	1.481	0.979–2.239	0.075
	CC	1.058	0.604–1.852	0.887	CC	0.577	0.257–1.299	0.234	CC	1.463	0.594–3.603	0.496
rs2764264	TT	1			TT	1			TT	1		
	TC	1.219	0.918–1.621	0.192	TC	1.085	0.733–1.608	0.690	TC	1.401	0.925–2.122	0.115
	CC	1.017	0.581–1.781	1.000	CC	0.628	0.283–1.393	0.323	CC	1.729	0.753–3.967	0.219
rs2802292	TT	1			TT	1			TT	1		
	TG	1.200	0.903–1.594	0.219	TG	1.092	0.737–1.618	0.689	TG	1.356	0.896–2.051	0.172
	GG	1.183	0.681–2.056	0.576	GG	1.069	0.497–2.296	1.000	GG	1.327	0.596–2.958	0.545
rs13220810	TT	1			TT	1			TT	1		
	TC	0.899	0.655–1.232	0.520	TC	0.725	0.464–1.133	0.174	TC	1.114	0.710–1.746	0.649
	CC	0.812	0.436–1.510	0.531	CC	0.715	0.304–1.681	0.519	CC	0.938	0.379–2.324	1.000

SNPs – single-nucleotide polymorphisms; OR – odds ratio; CI – confidence interval; BMI – body mass index. * Major allele homozygotes for each SNP were used as the reference group for calculation of OR. Significant *P* values are shown in bold.

cancers and aging diseases [13,15,16]. In a healthy long-lived Chinese population, rs2802288, rs2802292, and moderate physical activity were associated with a lower fasting hyperglycemia risk, lower levels of hyperglycemia (FPG and HbA1c) compared to middle-aged cohorts [9]. Nair et al. did not find an association with *FOXO3* and T2DM in a middle-aged South Indian Dravidian population; however, they found that rs2802288, which was in complete linkage disequilibrium with rs2802292, seemed to have lower FPG, indicating improved insulin resistance [30].

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 6. Association of the four candidate SNP variant genotypes with clinical characteristics.

SNPs		n	Age	BMI	HDL	LDL	TG	TC
rs1321779795	TT	216	68.27±6.66	25.51±2.87	1.22±0.28	3.03±0.87	2.09±1.69	5.25±1.06
	TC	186	66.85±6.47	25.25±3.09	1.19±0.29	3.03±0.89	1.80±1.04	5.13±1.05
	CC	22	69.64±7.01	25.79±2.60	1.16±0.17	2.94±0.75	1.59±0.82	4.90±0.82
	P		**0.039**	0.589	0.454	0.886	0.067	0.221
rs2764264	TT	218	68.32±6.65	25.55±2.89	1.22±0.28	3.02±0.87	2.09±1.69	5.24±1.06
	TC	178	66.89±6.51	25.16±3.07	1.19±0.29	3.04±0.88	1.80±1.04	5.14±1.05
	CC	27	69.04±6.68	25.77±2.64	1.16±0.20	2.84±0.82	1.63±0.99	4.83±0.92
	P		**0.06**	0.377	0.358	0.528	0.067	0.137
rs2802292	TT	215	68.29±6.51	25.58±2.92	1.22±0.28	3.01±0.88	2.08±1.69	5.22±1.07
	TG	178	66.88±6.73	25.12±3.06	1.20±0.29	3.05±0.87	1.81±1.06	5.16±1.03
	GG	30	68.80±6.37	25.93±2.42	1.14±0.19	2.84±0.78	1.67±0.93	4.83±0.87
	P		0.072	0.209	0.372	0.467	0.089	0.153
rs13220810	TT	301	67.67±6.54	25.46±3.06	1.20±0.29	2.99±0.88	1.97±1.45	5.16±1.05
	TC	103	67.6±6.74	25.03±2.56	1.21±0.26	3.11±0.84	1.85±1.37	5.20±1.07
	CC	20	69.4±7.49	26.84±2.80	1.27±0.28	2.98±0.85	1.84±0.98	5.17±1.02
	P		0.514	0.053	0.520	0.486	0.719	0.933

SNPs		n	ALT'	Cr	SBP	DBP	GLU	HbAlc	Hgb
rs1321779795	TT	216	22.95±12.01	62.00±13.85	149.02±20.13	83.09±11.14	8.96±1.96	7.77±1.36	145.70±14.622
	TC	186	23.12±13.44	65.35±50.92	150.39±22.01	83.95±11.14	8.55±1.71	7.52±1.11	143.13±14.08
	CC	22	24.32±18.68	63.66±16.29	142.30±21.99	78.65±9.15	8.29±1.03	7.49±0.76	146.05±12.37
	P		0.896	0.638	0.263	0.126	**0.04**	0.179	0.178
rs2764264	TT	218	22.88±11.94	61.86±13.73	149.47±20.71	83.22±11.25	8.99±2.01	7.78±1.36	145.51±14.51
	TC	178	23.25±13.59	65.62±51.88	149.54±21.37	83.62±10.98	8.54±1.64	7.49±1.10	143.17±14.17
	CC	27	23.74±17.08	64.54±16.00	144±21.42	80.46±10.59	8.35±1.13	7.70±0.88	147.22±11.51
	P		0.928	0.568	0.464	0.430	**0.025**	0.115	0.167
rs2802292	TT	215	22.33±11.71	62.13±13.72	149.59±21.32	83.49±11.35	8.98±2.03	7.77±1.38	145.89±14.36
	TG	178	24.05±13.89	65.69±51.88	149.67±21.12	83.32±10.97	8.57±1.64	7.54±1.11	142.98±14.61
	GG	30	23.30±16.40	62.62±16.12	144.82±19.35	80.21±9.71	8.15±0.95	7.50±0.80	145.52±11.13
	P		0.427	0.602	0.511	0.337	**0.015**	0.206	0.127
rs13220810	TT	301	23.72±14.08	64.53±40.63	149.95±22.04	83.02±11.41	8.72±1.75	7.68±1.19	144.14±14.86
	TC	103	21.28±9.77	61.73±16.02	146.02±16.58	83.54±10.09	8.81±2.00	7.69±1.17	146.14±12.25
	CC	20	23.10±10.18	60.25±13.60	157.67±24.65	84.61±11.68	8.86±2.15	6.79±2.18	143.80±15.41
	P		0.261	0.713	0.067	0.799	0.882	0.061	0.459

N – number; BMI – body mass index; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglyceride; TC – total cholesterol; ALT – glutamic-pyruvic transaminase; Cr – creatinine; SBP – systolic pressure; DBP – diastolic pressure; GLU – glucose; HgB – hemoglobin; HbAlc – glycosylated hemoglobin. Significant *P* values are shown in bold. Except the data for number, data are expressed as mean ± s.e. Unpaired t-test and ANOVA were used to compare the difference between groups. Note that *P* was the result from the total participate.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 7. The effect of FOXO3 genotype on glucose in hyperglycemia females and males.

Sex	Genotype	Change in glucose (mmol/L)*	95%CI	*P*
rs13217795				
Female	TC	–0.724	(–1.23 to –0.22)	**0.005**
n=213	CC	–1.093	(–1.78 to –0.41)	**0.03**
Male	TC	–0.092	(–0.62 to –0.44)	0.73
n=210	CC	–0.302	(–1.11 to –0.51)	0.44
rs2764264				
Female	TC	–0.801	(–1.30 to –0.30)	**0.002**
n=212	CC	–1.212	(–1.88 to –0.54)	**0.001**
Male	TC	–0.094	(–0.63 to 0.45)	0.732
n=211	CC	–0.180	(–0.93 to 0.57	0.624
rs2802292				
Female	TC	–0.754	(–1.26 to –0.25)	**0.004**
n=212	CC	–1.150	(–1.80 to –0.50)	**0.001**
Male	TC	–0.066	(–0.61 to 0.48)	0.811
n=211	CC	–0.543	(–1.18 to 0.10)	0.093
rs13220810				
Female	TC	0.401	(–0.34 to 1.14)	0.284
n=212	CC	0.806	(–1.19 to 2.80)	0.386
Male	TC	–0.178	(–0.71to 0.36)	0.510
n=212	CC	–0.540	(–1.35 to 0.27)	0.175

Significant *P* values are shown in bold. * Effect is compared to the major allele homozygotes of each SNP.

Females usually live longer than males in the world, which is independent of culture or socioeconomic status [37,38]. Females also have a longer life expectancy in the animal kingdom, such as *Drosophila* and *Caenorhabditis. Elegans* species [39,40]. However, causes of gender difference in life expectancy largely remained unclear, and this pervasive inequality has intrigued researchers for decades. There are more than 190 million women with diabetes worldwide, and the number is expected to rise to 313 million by 2040 [41]. In China, the prevalence of diabetes among women was 11.0% (95%CI, 10.7–11.4%) [2]. Since hyperglycemia was a major risk factor for a series of age-related diseases, such as myocardial infarction, stroke, chronic kidney disease, and even cancer [2,42–44], our findings suggested a possible protective mechanism by which *FOXO3* might contribute to the lifespan extension in women.

FOXO3, an evolutionarily conserved transcription factor in the insulin/insulin-like growth factor-1 signaling pathway, is expressed in major insulin sensitive tissues and insulin producing cells, which indicates *FOXO3* may play an important role in glucose regulation [19–21]. *Daf-16*, a *foxo3* homologue in *C. elegans*, regulates the transcription of genes which are involved in the regulation of glucose metabolism and longevity [45]. Its binding sites have been identified in the promoters of key genes like G6Pase and PEPCK [19]. A twin cohort study revealed the *FOXO3* rs2800292 G allele improved insulin sensitivity and increased *FOXO3* expression in skeletal muscle in a small Danish study [45]. *FOXO3* knockout mice had a lower rate of glucose uptake in glucose tolerant tests [31]. *FOXO3* is a housekeeping gene, whose missense mutation might cause severe phenotypes and premature death. Thus, all the variants found in *FOXO3* was noncoding variants [28]. This could partly explain why we find it might play an important role in sub-clinical traits rather than in disease itself.

Furthermore, the effect of *FOXO3* variants on diabetes may be a long-term process. The pathogenesis of T2D is closely associated with inflammatory processes, and cellular stress which may cause T2D which also induces or exacerbates inflammatory responses [46]. IL-1β induces apoptosis of pancreatic beta cells, and IL-1β production is suppressed by glyburide which is used to treat T2D. Production of inflammatory cytokines is reduced by *FOXO3* [47], and inflammatory responses are inhibited by rs12212067 via TGFβ1, which has tight linkage disequilibrium

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

with rs2802292 [48]. This protection effect of *FOXO3* variants in diabetes is worth further investigation.

Females with diabetes had a higher risk of all-cause mortality, cardiovascular disease hospitalizations, and all-site cancer than males with diabetes [49,50]. The mechanism underlying these differences is largely unclear. Estrogen plays a role in enhancing insulin sensitivity, regulating glucose metabolism and lipid metabolism in the female body, improving vascular endothelial dysfunction and reducing blood pressure. To a certain extent, hormonal changes affect the control of diabetes in menopausal females. Once estrogen level change, especially when there is a decline, coupled with diabetes caused by a decrease in insulin secretion, these regulatory effects will be weakened or disappear, followed by the rapid occurrence of atherosclerosis, and a greatly increased risk of cardiovascular disease [51]. Studies have shown that women with diabetes who had accepted hormone replacement therapy had better glucose metabolism and were more energetic [52,53]. As mentioned, *FOXO3* is tightly associated with diabetes. Intriguingly, it has been reported that *FOXO3* expressions and functions could be altered by estrogen [54]. Our study demonstrated that there was an association between rs13217795, rs2764264, and rs2802292 and blood glucose levels in females but not in males, which might suggest a new mechanism for estrogen action in diabetes development.

Conclusions

In summary, our data showed longevity-associated variants in *FOXO3* might reduce blood glucose levels in Chinese elderly women with T2D, but not in men. This might provide a new insight to individual patient management of hyperglycemia in the elderly. The controls for this study were recruited from the population who received routine physical examination in Shanghai hospitals, and they were not hospitalized patients. Thus, they may be representative of the general population even though selection bias cannot be ruled out completely. However, studies with a larger sample size and evaluation of postprandial blood sugar values are needed to further validate our study finding.

Ethics approval and consent to participate

The ethics committee of Minhang Hospital approved this study and all participants gave informed consent to participate in the study.

Conflict of interest

None.

References:

1. Defronzo RA: Banting Lecture. From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. Diabetes, 2009; 58: 773–95
2. Xu Y, Wang L, He J et al:Prevalence and control of diabetes in Chinese adults. JAMA, 2013; 310: 948–59
3. Stumvoll M, Goldstein BJ, van Haeften TW: Type 2 diabetes: Principles of pathogenesis and therapy. Lancet, 2005; 365: 1333–46
4. Dupre ME, Liu G, Gu D: Predictors of longevity: Evidence from the oldest old in China. Am J Public Health, 2008; 98: 1203–8
5. Kim-Muller JY, Zhao S, Srivastava S et al: Metabolic inflexibility impairs insulin secretion and results in MODY-like diabetes in triple FoxO-deficient mice. Cell Metabolism, 2014; 20: 593–602
6. Stamler J, Vaccaro O, Neaton JD et al: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care, 1993; 16: 434–44
7. Wei M, Gaskill SP, Haffner SM et al: Effects of diabetes and level of glycemia on all-cause and cardiovascular mortality. The San Antonio Heart Study. Diabetes Care, 1998; 21: 1167–72
8. Florez H, Ma Y, Crandall JP et al: Parental longevity and diabetes risk in the Diabetes Prevention Program. J Gerontol A Biol Sci Med Sci, 2011; 66: 1211–17
9. Sun L, Hu C, Qian Y et al: Age-based differences in the genetic determinants of glycemic control: A case of FOXO3 variations. PLoS One, 2015; 10: e0126696
10. Ponugoti B, Dong G, Graves DT: Role of forkhead transcription factors in diabetes-induced oxidative stress. Exp Diabetes Res, 2012; 2012: 939751
11. Consoli A: Role of liver in pathophysiology of NIDDM. Diabetes Care, 1992; 15: 430–41
12. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM. A balanced overview. Diabetes Care, 1992; 15: 318–68
13. Nho RS, Hergert P: FoxO3a and disease progression. World J Biol Chem, 2014; 5: 346–54
14. Huang H, Tindall DJ: Dynamic FoxO transcription factors. J Cell Sci, 2007; 120: 2479–87
15. Warr MR, Binnewies M, Flach J et al: FOXO3A directs a protective autophagy program in haematopoietic stem cells. Nature, 2013; 494: 323–27
16. Arden KC: Multiple roles of FOXO transcription factors in mammalian cells point to multiple roles in cancer. Exp Gerontol, 2006; 41: 709–17
17. Morris BJ, Chen R, Donlon TA et al: Association analysis of FOXO3 longevity variants with blood pressure and essential hypertension. Am J Hypertens, 2016; 29: 1292–300
18. Willcox BJ, Donlon TA, He Q et al: FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci USA, 2008; 105: 13987–92
19. Onuma H, Vander Kooi BT, Boustead JN et al: Correlation between FOXO1a (FKHR) and FOXO3a (FKHRL1) binding and the inhibition of basal glucose-6-phosphatase catalytic subunit gene transcription by insulin. Mol Endocrinol, 2006; 20: 2831–47
20. Luo W, Cao J, Li J et al: Adipose tissue-specific PPARgamma deficiency increases resistance to oxidative stress. Exp Gerontol, 2008; 43: 154–63
21. Williamson DL, Raue U, Slivka DR et al: Resistance exercise, skeletal muscle FOXO3A, and 85-year-old women. J Gerontol A Biol Sci Med Sci, 2010; 65: 335–43
22. 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
23. Bao JM, Song XL, Hong YQ et al: Association between FOXO3A gene polymorphisms and human longevity: A meta-analysis. Asian J Androl, 2014; 16: 446–52
24. Broer L, Buchman AS, Deelen J et al: GWAS of longevity in CHARGE consortium confirms APOE and FOXO3 candidacy. J Gerontol A Biol Sci Med Sci, 2015; 70: 110–18

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

25. 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: 2700–5
26. 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: 4897–904
27. Pawlikowska L, Hu D, Huntsman S et al: Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. Aging Cell, 2009; 8: 460–72
28. 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: 1132–39
29. 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: 1010–17
30. Nair AK, Sugunan D, Kumar H et al: Association analysis of common variants in FOXO3 with type 2 diabetes in a South Indian Dravidian population. Gene, 2012; 491: 182–86
31. Castrillon DH, Miao L, Kollipara R et al: Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. Science, 2003; 301: 215–18
32. Daumer C, Flachsbart F, Caliebe A et al: Adjustment for smoking does not alter the FOXO3A association with longevity. Age, 2014; 36: 911–21
33. Di Bona D, Accardi G, Virruso C et al: Association between genetic variations in the insulin/insulin-like growth factor (Igf-1) signaling pathway and longevity: A systematic review and meta-analysis. Curr Vasc Pharmacol, 2014; 12: 674–81
34. Li N, Luo H, Liu X et al: Association study of polymorphisms in FOXO3, AKT1 and IGF-2R genes with human longevity in a Han Chinese population. Oncotarget, 2016; 7: 23–32
35. Revelas M, Thalamuthu A, Oldmeadow C et al: Review and meta-analysis of genetic polymorphisms associated with exceptional human longevity. Mech Ageing Dev, 2018; 175: 24–34
36. Sun L, Hu C, Zheng C et al: FOXO3 variants are beneficial for longevity in Southern Chinese living in the Red River Basin: A case-control study and meta-analysis. Sci Rep, 2015; 5: 9852
37. Zarulli V, Barthold Jones JA, Oksuzyan A et al: Women live longer than men even during severe famines and epidemics. Proc Natl Acad Sci USA, 2018; 115: E832–40
38. Barford A, Dorling D, Davey Smith G et al: Life expectancy: Women now on top everywhere. BMJ, 2006; 332: 808
39. Gendron CM, Kuo TH, Harvanek ZM et al: Drosophila life span and physiology are modulated by sexual perception and reward. Science, 2014; 343: 544–48
40. Berman JR, Kenyon C: Germ-cell loss extends *C. elegans* life span through regulation of DAF-16 by kri-1 and lipophilic-hormone signaling. Cell 2006;124: 1055-1068.
41. Ogurtsova K, da Rocha Fernandes JD, Huang Y et al: IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract, 2017; 128 40–50
42. Danaei G, Lawes CM, Vander Hoorn S et al: Global and regional mortality from ischaemic heart disease and stroke attributable to higher-than-optimum blood glucose concentration: comparative risk assessment. Lancet, 2006; 368: 1651–59
43. Levey AS, Coresh J: Chronic kidney disease. Lancet, 2012; 379: 165–80
44. Larsson SC, Orsini N, Wolk A: Diabetes mellitus and risk of colorectal cancer: A meta-analysis. J Natl Cancer Inst, 2005; 97: 1679–87
45. Banasik K, Ribel-Madsen R, Gjesing AP et al: The FOXO3A rs2802292 G-allele associates with improved peripheral and hepatic insulin sensitivity and increased skeletal muscle-FOXO3A mRNA expression in twins. J Clin Endocrinol Metab, 2011; 96: E119–24
46. Masters SL, Dunne A, Subramanian SL et al: Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes. Nat Immunol, 2010; 11: 897–904
47. Dejean AS, Beisner DR, Ch'en IL et al: Transcription factor Foxo3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. Nat Immunol, 2009; 10: 504–13
48. Lee JC, Espeli M, Anderson CA et al: Human SNP links differential outcomes in inflammatory and infectious disease to a FOXO3-regulated pathway. Cell, 2013; 155: 57–69
49. Roche MM, Wang PP: Sex differences in all-cause and cardiovascular mortality, hospitalization for individuals with and without diabetes, and patients with diabetes diagnosed early and late. Diabetes Care, 2013; 36: 2582–90
50. Ohkuma T, Peters SAE, Woodward M: Sex differences in the association between diabetes and cancer: A systematic review and meta-analysis of 121 cohorts including 20 million individuals and one million events. Diabetologia, 2018; 61: 2140–54
51. Gupte AA, Pownall HJ, Hamilton DJ: Estrogen: An emerging regulator of insulin action and mitochondrial function. J Diabetes Res, 2015; 2015: 916585
52. Salpeter SR, Walsh JM, Ormiston TM et al: Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes Metab, 2006; 8: 538–54
53. Margolis KL, Bonds DE, Rodabough RJ et al: Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: Results from the Women's Health Initiative Hormone Trial. Diabetologia, 2004; 47: 1175–87
54. Thompson MG, Peiffer DS, Larson M et al: FOXO3, estrogen receptor alpha, and androgen receptor impact tumor growth rate and infiltration of dendritic cell subsets differentially between male and female mice. Cancer Immunol Immunother, 2017; 66: 615–25

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]