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

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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
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) and 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%.

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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 (HbA1c) 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 (HbG) 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 χ² 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±1.3/8.54±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).
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 (P<0.05).

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

![Figure 1](image1.png)  
Figure 1. Meta-analysis of the association between rs2802292 and longevity. CI – confidence interval; OR – odds ratio.

![Figure 2](image2.png)  
Figure 2. Meta-analysis of the association between rs13217795 and longevity. CI – confidence interval; OR – odds ratio.

![Figure 3](image3.png)  
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.
### 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)               | 67.7 ± 6.61      | 66.8 ± 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/m²)               | 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 ± 6.66     | 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 |
| HbA1c (%)                 | 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; HbA1c – 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    |
|-----------|-----|-----|-----|-----|-----|------|
| All       | 448 | 332 | 55  | 1228| 442 | 0.59 |
| Case      | 218 | 178 | 27  | 614 | 232 | 0.27 |
| Control   | 230 | 154 | 28  | 614 | 210 | 0.8  |
| rs1321779S| All | 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 | 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.
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            | 122 |       |       |
| TC            | 121 |       |       |
| CC            | 132 |       |       |
| χ²            |     |         |       |
| 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    |       |       |     |     |   |       |       |     |     |     |       |       |
| rs2764264     |     |         |       |
| TT            | 122 |       |       |
| TC            | 121 |       |       |
| CC            | 132 |       |       |
| χ²            |     |         |       |
| P             |     |         |       |
| Diabetes      | 216 | 186    | 22    | 1.93  | 0.381 | 106 | 95  | 11 | 1.809 | 0.405 | 112 | 83 | 16 | 3.563 | 0.168 |
| Controls      | 237 | 151    | 28    |       |       |     |     |   |       |       |     |     |     |       |       |
| rs2802292     |     |         |       |
| TT            | 122 |       |       |
| TG            | 121 |       |       |
| GG            | 132 |       |       |
| χ²            |     |         |       |
| 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    |       |       |     |     |   |       |       |     |     |     |       |       |
| rs13220810    |     |         |       |
| TT            | 122 |       |       |
| TC            | 121 |       |       |
| CC            | 132 |       |       |
| χ²            |     |         |       |
| 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    |       |       |     |     |   |       |       |     |     |     |       |       |

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 |
|---------------|-----|---------|-------|
| rs13217795    |     |         |       |
| TT            | 1*  |         |       |
| TC            | 1.352 |       | 1.018–1.794 | 0.037 |       |
| CC            | 1.058 | 0.604–1.852 | 0.887 |       |
| χ²            |     |         |       |
| P             |     |         |       |
| Diabetes      | 216 | 186    | 22    |       |       |     |     |   |       |       |     |     |     |       |       |
| Controls      | 237 | 151    | 28    |       |       |     |     |   |       |       |     |     |     |       |       |
| rs2764264     |     |         |       |
| TT            | 1   |         |       |
| TC            | 1.219 |       | 0.918–1.621 | 0.192 |       |
| CC            | 1.017 | 0.581–1.781 | 1.000 |       |
| χ²            |     |         |       |
| P             |     |         |       |
| Diabetes      | 215 | 178    | 30    |       |       |     |     |   |       |       |     |     |     |       |       |
| Controls      | 229 | 158    | 27    |       |       |     |     |   |       |       |     |     |     |       |       |
| rs2802292     |     |         |       |
| TT            | 1   |         |       |
| TG            | 1.200 |       | 0.903–1.594 | 0.219 |       |
| GG            | 1.183 | 0.681–2.056 | 0.576 |       |
| χ²            |     |         |       |
| P             |     |         |       |
| Diabetes      | 215 | 178    | 30    |       |       |     |     |   |       |       |     |     |     |       |       |
| Controls      | 229 | 158    | 27    |       |       |     |     |   |       |       |     |     |     |       |       |
| rs13220810    |     |         |       |
| TT            | 1   |         |       |
| TG            | 1.099 |       | 0.733–1.608 | 0.690 |       |
| GG            | 1.108 | 0.733–1.806 | 0.590 |       |
| χ²            |     |         |       |
| P             |     |         |       |
| Diabetes      | 301 | 103    | 20    |       |       |     |     |   |       |       |     |     |     |       |       |
| Controls      | 281 | 107    | 23    |       |       |     |     |   |       |       |     |     |     |       |       |

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.
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 | 223           | 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 | 2.09±1.69 | 5.22±1.07 |
|            | TC | 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    | 0.317    | 0.670    | 0.180    | 0.080    | 0.53      |
| 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    |

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.
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.

### 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                           | −0.060 | (−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 | (−0.71 to 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.
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.

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