The role of FOXO3 polymorphisms in susceptibility to tuberculosis in a Chinese population

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Abstract

Background: Tuberculosis (TB) is a significant worldwide health problem, and is caused by Mycobacteria tuberculosis. Recent studies have suggested that FOXO3 plays vital roles in the risk of immune–related infectious diseases such as TB.

Methods and Results: The present study aimed to evaluate FOXO3 genetic variants and TB risk. We recruited 510 TB patients and 508 healthy controls in this study. All subjects were genotyped with the Agena MassARRAY platform. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression adjusted for age and gender. Our result revealed that rs3800229 T/G and rs4946935 G/A genotypes significantly increased the risk of TB (OR = 1.34, 95% CI = 1.04–1.74, p = 0.026; OR = 1.34, 95% CI = 1.03–1.73, p = 0.029, respectively). In stratified analysis according to gender and age, we observed that rs3800229 T/G and rs4946935 G/A genotypes were associated with an increase the risk of TB among males and age ≤41 years, respectively (OR = 1.47, 95% CI = 1.06–2.04, p = 0.022 and OR = 1.45, 95% CI = 1.05–2.02, p = 0.025).

Conclusions: Our study showed that rs3800229 and rs4946935 in FOXO3 were associated with a risk of TB in the Chinese population.

KEYWORDS
FOXO3, infectious diseases, Mycobacteria tuberculosis, polymorphism

Introduction

Tuberculosis (TB), as one of the most common chronic infectious disease, is caused by Mycobacteria tuberculosis and remains high morbidity and mortality in our world. Among those infected with Mycobacterium tuberculosis, approximately 5%–15% develop clinical disease during their lifetime (Chen, Liu, Zhu, Yang, & Lu, 2013). It is reported that there are about 10.0 million new TB cases and 1.3 million deaths occurred in 2017 (WHO. Global tuberculosis report 2018). Studies suggest that host genetic variants play a crucial role in determining resistance or susceptibility to TB disease (Liu et al., 2015; Rodriguez-Castillo et al., 2015). However, the molecular mechanism of the genetic predisposition to TB is still largely unknown.
Recently, several candidate TB-susceptibility genes have been identified, including Vitamin D receptor gene (Sun et al., 2015), CYP7A1 (Qrafli et al., 2014), CD14 (Miao, Ge, Xu, & Xu, 2014), TLR9 (Bharti et al., 2014) and SEC14L2 (Du et al., 2014), TLR9 (Hosaka et al., 2004).

FOXO3 is known to play a role in modulating immune response and in response to growth factors (Hedrick, Hess Michelini, Doedens, Goldrath, & Stone, 2012). Several SNPs were found to be associated with increased lifespan in the German and Han Chinese population (Flachsbart et al., 2017; Sun et al., 2015). In addition, in the immune system, it has been reported that FOXO3 is an important regulator of T cell-, dendritic cell- and macrophage- homeostasis (Dejean et al., 2009; Watkins & Hurwitz, 2012). Previous genome-wide association studies have shown that FOXO3 gene variants were observed to be associated with severity of autoimmune diseases and infectious diseases, such as Crohn’s disease and malaria (Lee et al., 2013; Marlow, Han, Triggs, & Ferguson, 2015). However, there were few reports regarding the impact of FOXO3 genetic variants polymorphism on susceptibility to TB (Lu et al., 2016).

Thus, in this study, our primary objective was to evaluate the association between FOXO3 genetic variants and the risk of TB in a sample of Chinese population.

2 | MATERIALS AND METHODS

2.1 | Study populations

In this study, we recruited 510 TB patients and 508 age- and sex-matched healthy controls from Xi’an Chest Hospital, Shaanxi Province. All TB patients and healthy controls were unrelated to the Han Chinese population. Patients were diagnosed by specialized doctors according to clinical signs, chest x-ray examination and positive sputum smear for acid-fast bacilli. All healthy controls underwent an x-ray examination, none of them showed any clinical characteristics of TB. TB patients and healthy controls, who were HIV positive, autoimmune diseases, chronic inflammatory or other diseases affecting host immunity were excluded. Blood samples were obtained from each subject for molecular analysis.

2.2 | Genotyping

Genomic DNA was extracted from whole blood samples with GoldMag whole blood genomic DNA purification kit (GoldMag Co. Ltd. Xi’an city, China). DNA concentration was evaluated with a NanoDrop 2000 platform (Thermo Fisher S 95% Clenicient, Waltham, MA, USA), according to the manufacturer’s instruction. The genes associated with TB were selected from the Genomic Wide Association Study using UCSC (http://genome.ucsc.edu/) database. We found that FOXO3 was associated with several diseases including TB (Greer & Brunet, 2008). The candidate SNPs of FOXO3 were selected from the 1,000 Genomes Project data (http://www.internationalgenome.org/) with minor allele frequency (MAF) > 0.02 in the Asian population and MAF > 0.05 in the Chinese Han Beijing population. Finally, four SNPs in FOXO3 were selected in this case-control study. The Agena Bioscience Assay Design Suite V2.0 software (https://agena.com/online-tools/) was performed to analyze the amplification and extension primers. The MassARRAY Nanodispenser and MassARRAY iPLEX platform (both from Agena Bios 95% Clence, San Diego, CA, USA) were used to genotype the SNPs. Data management was performed using Agena Bioscience TYPER version 4.0 software as previously described (Zhu et al., 2017).

2.3 | Statistical analysis

The base characteristics such as age and gender of TB patients and healthy controls were conducted using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software. Further, the following analysis was performed through PLINK software (version 1.07). Deviation from Hardy-Weinberg equilibrium (HWE) was assessed using the Chi-square test to compare the observed and expected genotype frequencies among the control subjects. Allele and genotype frequencies were compared between TB patients and healthy controls using the χ² test and Fisher’s exact test. To evaluate the association between FOXO3 SNPs and the risk of TB, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) adjustment gender and age. We also applied four different genetic models: the codominant model, the dominant model, the recessive model and the log-additive model to assess FOXO3 SNPs associations with TB risk using PLINK software (version 1.07). Power and Sample Size Calculation software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize) were used to calculate the power of the significant difference. The haplotypes of the candidate SNPs were analyzed using Haploview v4.2. Linkage disequilibrium (LD) between SNPs was analyzed using the pairwise LD measure D’. Pairwise D’ > 0.80 suggested that the SNPs were in high LD. For all statistical tests, p < 0.05 was considered statistically significant.

3 | RESULTS

A total of 1,018 individuals including 510 TB patients (mean age, 41.90 ± 14.83 years old; 318 males and 192 females) and 508 healthy controls (mean age, 41.14 ± 18.42 years old; 316 males and 192 females) were recruited in this study. There was no significant difference among the groups regarding age (p = 0.469) and gender (p = 0.961). The general demographic characteristics are shown in Table 1.

Detailed SNPs information including the position, MAF and HWE p value are listed in Table 2. The MAF of all SNPs
were more than 5% in our study population. All candidate SNPs accorded with HWE ($p > 0.05$).

Differences in SNPs genotype and allele frequencies distribution between TB patients and healthy controls were analyzed using Chi‐squared test and ORs to assess the associations with TB risk, as displayed in Table 3. The minor allele of each SNP as a risk factor was compared to the wild‐type allele. However, we had not observed that any SNPs were significantly different of allele frequencies between cases and healthy controls (Table 3). Further, different genetic models were performed to evaluate the potential association between the $FOXO3$ polymorphism and the risk of TB after adjusting age and gender. We observed that rs3800229 T/G genotype was associated with increased the risk of TB in comparison to the common genotype TT ($OR = 1.34$, 95% CI = 1.04–1.74, $p = 0.026$), with power values of 0.639. Similarly, compared with the common genotype G/G, the genotype G/A of rs4946935 was significantly associated with TB risk ($OR = 1.34$, 95% CI = 1.03–1.73, $p = 0.029$, power = 0.639). However, no significant correlation was identified between rs3800229 and rs4946935 genotypes and TB risk in the dominant model, recessive model and log–additive model.

Moreover, in accordance with the stratified analyses by gender and age. We found that the T/G genotype of rs3800229 and the G/A genotype of rs4946935 were associated with increased risk of TB in males ($OR = 1.47$, 95% CI = 1.06–2.04, $p = 0.022$; $OR = 1.45$, 95% CI = 1.05–2.02, $p = 0.025$), respectively) with power values of 0.825 and 0.798, respectively. There was no significant association between rs3800229 and rs4946935 genotypes and TB risk in females (Table 4). Besides, the distribution frequency of rs3800229 T/G and T/G—G/G genotype were higher in TB risk with age ≤41 years ($OR = 1.55$, 95% CI = 1.07–2.26, $p = 0.020$, power = 0.929; $OR = 1.44$, 95% CI = 1.01–2.06, $p = 0.044$, power = 0.817, respectively). Our results also suggested that rs4946935 G/A and G/A—A/A genotypes were significantly associated with TB risk with age ≤41 years ($OR = 1.55$, 95% CI = 1.07–2.26, $p = 0.020$, power = 0.929 and $OR = 1.44$, 95% CI = 1.01–2.06, $p = 0.044$, power = 0.817, respectively). No association of rs3800229 and rs4946935 genotype frequencies and TB risk were observed with age > 41 years. (Table 5).

Subsequently, the association of $FOXO3$ with TB susceptibility was explored through haplotype analysis. LD analysis confirmed that SNPs rs12212067, rs3800229 and rs4946935 compose an LD block, as shown in Figure 1. Pairwise D′ value between SNPs rs12212067 and rs3800229 was 0.99. Pairwise D′ value between SNPs rs12212067 and rs4946935 was 0.99. Pairwise D′ value between SNPs rs3800229 and rs4946935 was 1.00. The results indicated that these SNPs were high in LD (all pairwise D′ > 0.80). To examine the effect of haplotype on the risk of TB, the haplotype‐based logical regression method adjusted by age and gender was performed. However, our results did not reveal a significant correlation between common haplotype and TB risk (all $p > 0.05$). The result is presented in Table 6.

### Discussion

Previous studies confirmed that host genetic variants play an important role in TB infection and progress, and several candidate genes have been identified (Du et al., 2017; Qrafli et al., 2014; Sun et al., 2015). However, the association between $FOXO3$ variations polymorphism and TB risk is poorly understood. In this study, we investigated the relationship of $FOXO3$ variants polymorphism and TB risk. The result suggested that the T to G mutation of rs3800229 and the G to A mutation of rs4946935 significantly increased the risk of TB in the Chinese population.
**TABLE 3** The association between four SNPs within FOXO3 and the risk of tuberculosis

| SNP       | Model       | Genotype | TB (n %)  | Controls (n %) | Adjusted by gender and age OR (95% CI) p | Crude analysis OR (95% CI) p |
|-----------|-------------|----------|-----------|----------------|-----------------------------------------|-----------------------------|
| rs1536057 | Allele      | G        | 838 (82.16) | 852 (83.86)    | 1.13 (0.90–1.42) 0.307                  | Baseline                   |
|           |             | A        | 182 (17.84) | 164 (16.14)    |                                          |                             |
|           | Codominant  | G/G      | 340 (66.67) | 360 (70.87)    | Baseline                                | 1.27 (0.96–1.67) 0.091     |
|           |             | G/A      | 158 (30.98) | 132 (25.98)    | 1.28 (0.97–1.68) 0.083                  |                             |
|           |             | A/A      | 12 (2.35)   | 16 (3.15)      | 0.79 (0.37–1.69) 0.538                  |                             |
|           | Dominant    | G/G      | 340 (66.67) | 360 (70.87)    | Baseline                                | 1.22 (0.93–1.59) 0.149     |
|           |             | G/A—A/A  | 170 (33.33) | 148 (29.13)    | 1.22 (0.94–1.59) 0.140                  |                             |
|           | Recessive   | G/G—G/A  | 498 (97.65) | 492 (96.85)    | Baseline                                | 0.74 (0.35–1.58) 0.439     |
|           |             | A/A      | 12 (2.35)   | 16 (3.15)      | 0.73 (0.34–1.57) 0.424                  |                             |
|           | Log–additive|        | –          | –              | 1.13 (0.90–1.43) 0.295                  | 1.13 (0.90–1.43) 0.305     |
| rs12212067| Allele      | T        | 887 (86.96) | 894 (87.99)    | 1.10 (0.84–1.43) 0.482                  |                             |
|           |             | G        | 133 (13.04) | 122 (12.01)    |                                          |                             |
|           | Codominant  | T/T      | 385 (75.49) | 396 (77.95)    | Baseline                                | 1.18 (0.87–1.59) 0.280     |
|           |             | T/G      | 117 (22.94) | 102 (20.08)    | 1.19 (0.88–1.61) 0.261                  |                             |
|           |             | G/G      | 8 (1.57)    | 10 (1.97)      | 0.82 (0.32–2.09) 0.673                  |                             |
|           | Dominant    | T/T      | 385 (75.49) | 360 (77.95)    | Baseline                                | 1.15 (0.86–1.54) 0.353     |
|           |             | T/G—G/G  | 125 (24.51) | 148 (22.05)    | 1.16 (0.86–1.55) 0.334                  |                             |
|           | Recessive   | T/T—T/G  | 502 (98.43) | 498 (98.03)    | Baseline                                | 0.79 (0.31–2.03) 0.629     |
|           |             | G/G      | 8 (1.57)    | 10 (1.97)      | 0.79 (0.31–2.01) 0.617                  |                             |
|           | Log–additive|        | –          | –              | 1.10 (0.85–1.43) 0.469                  | 1.10 (0.85–1.42) 0.486     |
| rs3800229 | Allele      | T        | 775 (75.98) | 789 (77.66)    | Baseline                                |                             |
|           |             | G        | 245 (24.02) | 227 (22.34)    | 1.10 (0.89–1.35) 0.370                  |                             |
|           | Codominant  | T/T      | 285 (55.88) | 311 (61.22)    | Baseline                                | 1.34 (1.03–1.74) 0.028°    |
|           |             | T/G      | 205 (40.20) | 167 (32.87)    | 1.34 (1.04–1.74) 0.026°                 |                             |
|           |             | G/G      | 20 (3.92)   | 30 (5.91)      | 0.73 (0.41–1.31) 0.294                  |                             |
|           | Dominant    | T/T      | 285 (55.88) | 311 (61.22)    | Baseline                                | 1.25 (0.97–1.60) 0.081     |
|           |             | T/G—G/G  | 225 (44.12) | 197 (38.78)    | 1.25 (0.97–1.60) 0.081                  |                             |
|           | Recessive   | T/T—T/G  | 490 (96.08) | 478 (94.09)    | Baseline                                | 0.65 (0.36–1.16) 0.146     |
|           |             | G/G      | 20 (3.92)   | 30 (5.91)      | 0.65 (0.37–1.17) 0.149                  |                             |
|           | Log–additive|        | –          | –              | 1.10 (0.90–1.36) 0.355                  | 1.10 (0.89–1.36) 0.364     |

(Continues)
**TABLE 3** (Continued)

| SNP       | Model           | Genotype | TB (n %) | Controls (n %) | Adjusted by gender and age | Crude analysis |
|-----------|-----------------|----------|----------|----------------|----------------------------|----------------|
| rs4946935 | Allele          | G        | 775 (75.98) | 790 (77.76) | Baseline                  |                |
|           |                 | A        | 245 (24.02) | 226 (24.24) | 1.11 (0.90–1.36)          | 0.342          |
|           | Codominant      | G/G      | 285 (55.88) | 311 (61.22) | Baseline                  | 1.33 (1.03–1.73) | 0.031* |
|           |                 | G/A      | 205 (40.20) | 168 (33.07) | 1.34 (1.03–1.73)          | 0.029*         |
|           |                 | A/A      | 20 (3.92)   | 29 (5.71)   | 0.75 (0.42–1.36)          | 0.351          |
|           | Dominant        | G/G      | 285 (55.88) | 311 (61.22) | Baseline                  | 1.25 (0.97–1.60) | 0.084 |
|           |                 | G/A—A/A | 225 (44.12) | 197 (38.78) | 1.25 (0.97–1.60)          | 0.081          |
|           | Recessive       | G/G—G/A | 490 (96.08) | 479 (94.29) | Baseline                  | 0.67 (0.38–1.21) | 0.185 |
|           |                 | A/A      | 20 (3.92)   | 29 (5.71)   | 0.68 (0.38–1.21)          | 0.188          |
|           | Log–additive    | –        | –         | –            | 1.11 (0.90–1.37)          | 0.327          |

Abbreviations: CI, confidence interval; OR, odds ratio; SNP: single nucleotide polymorphism.

*p < 0.05 indicates statistical significance.

**TABLE 4** The association between two SNPs within FOXO3 and the risk of tuberculosis stratified by gender

| SNP       | Model           | Genotype | Male | Female |
|-----------|-----------------|----------|------|--------|
|           |                 |          | OR (95% CI) | p*  | OR (95% CI) | p*  |
| rs3800229 | Codominant      | T/T      | Baseline | | Baseline |
|           |                 | T/G      | 1.47 (1.06–2.04) | 0.022* | 1.14 (0.74–1.75) | 0.563 |
|           |                 | G/G      | 0.54 (0.24–1.22) | 0.139 | 1.06 (0.44–2.54) | 0.896 |
|           | Dominant        | T/T      | Baseline | | Baseline |
|           |                 | T/G—G/G | 1.33 (0.97–1.82) | 0.081 | 1.12 (0.75–1.69) | 0.576 |
|           | Recessive       | T/T—T/G | Baseline | | Baseline |
|           |                 | G/G      | 0.46 (0.21–1.04) | 0.062 | 1.01 (0.43–2.40) | 0.976 |
|           | Log–additive    | –        | 1.12 (0.85–0.85) | 0.417 | 1.08 (0.78–1.51) | 0.641 |

rs4946935

| SNP       | Model           | Genotype | Male | Female |
|-----------|-----------------|----------|------|--------|
|           |                 |          | OR (95% CI) | p*  | OR (95% CI) | p*  |
|           | Codominant      | G/G      | Baseline | | Baseline |
|           |                 | G/A      | 1.45 (1.05–2.02) | 0.025* | 1.14 (0.74–1.75) | 0.563 |
|           |                 | A/A      | 0.57 (0.25–1.30) | 0.179 | 1.06 (0.44–2.54) | 0.896 |
|           | Dominant        | G/G      | Baseline | | Baseline |
|           |                 | G/A—A/A | 1.33 (0.97–1.82) | 0.081 | 1.12 (0.75–1.69) | 0.576 |
|           | Recessive       | G/G—G/A | Baseline | | Baseline |
|           |                 | A/A      | 0.49 (0.22–1.11) | 0.086 | 1.01 (0.43–2.40) | 0.976 |
|           | Log–additive    | –        | 1.13 (0.86–0.86) | 0.378 | 1.08 (0.78–1.51) | 0.641 |

Abbreviations: CI, confidence interval; OR, odds ratio; SNP: single nucleotide polymorphism.

*p* < 0.05 indicates statistical significance.

FOXO transcription factor is a subfamily of the FOX family, which consists of four members including FOXO1, FOXO3, FOXO4 and FOXO6 (Hosaka et al., 2004). These transcription factors have a wide range of functions, such as cellular differentiation, cell–cycle control, oxidative stress, apoptosis and other cellular functions (Greer & Brunet, 2008; Maiese, Chong, Hou, & Shang, 2009). FOXO3 is 124.94 kb in length and located on chromosome 6q21. Increasing
evidence shows that FOXO3 plays an important role in the human immune system (Nguetse, Kremsner, & Velavan, 2015). It is involved in the regulation of several immune functions in dendritic and T cells as well as in macrophages (Dejean et al., 2009; Watkins & Hurwitz, 2012).

Recently, the studies confirmed that FOXO3 contributed to the pathogenesis of several immune associated disorder, including TB (Haoues et al., 2014; Lu et al., 2016). In the study of Lee et al, it was suggested that the minor G allele of rs12212067 in FOXO3 is associated with a milder course of Crohn’s disease, rheumatoid arthritis and with increased risk of severe malaria, which through down-regulation of proinflammatory cytokines including TNFα and up-regulation of anti-inflammatory cytokines including IL–10. (Junqueira et al., 2010; Langhorne et al., 2004; Lee et al., 2013). Sun et al revealed that the G allele of rs12212067 in FOXO3 might effect FOXO3 expression, subsequently affecting the TB progress in active TB patients (Lu et al., 2016). These researches indicate that maintenance of suitable FOXO3 levels is critical in preventing TB. The mutation of FOXO3 polymorphisms leads to change in the activity of the transcription factor and increases susceptibility for TB.

As we all know, such studies have confirmed that age and gender play a crucial role in the etiology of TB (Zhang et al., 2011). The social, environment and lifestyle factors have also been found to be associated with TB risk in the world (Murray, Oxlade, & Lin, 2011; Wang & Shen, 2009). Thus, persons may have different association with TB risk in different gender and age. In this study, our stratified analyses discover a significant effect of rs3800229 T/G and rs4946935 G/A genotypes on risk of TB among males and age ≤41 years, respectively.

#### TABLE 5
The association between two SNPs within FOXO3 and the risk of tuberculosis stratified by age

| SNP       | Model   | Genotype | ≤41 (OR 95% CI) | p*   | >41 (OR 95% CI) | p*   |
|-----------|---------|----------|-----------------|------|-----------------|------|
| rs3800229 | Codominant | T/T Baseline | 1.55 (1.07–2.26) | 0.020* | 1.05 (0.71–1.57) | 0.793 |
|           |         | T/G      | 0.89 (0.40–1.97) | 0.769 | 0.62 (0.24–1.62) | 0.331 |
|           | Dominant | T/T Baseline | 1.44 (1.01–2.06) | 0.044* | 1.00 (0.68–1.46) | 0.994 |
|           | Recessive | T/T – T/G Baseline | 0.75 (0.34–1.64) | 0.470 | 0.61 (0.24–1.56) | 0.305 |
|           | Log–additive | – | 1.23 (0.92–1.65) | 0.165 | 0.67 (0.26–1.76) | 0.420 |
| rs4946935 | Codominant | G/G Baseline | 1.55 (1.07–2.26) | 0.020* | 1.04 (0.70–1.54) | 0.843 |
|           |         | G/A      | 0.89 (0.40–1.97) | 0.769 | 0.68 (0.26–1.80) | 0.444 |
|           | Dominant | G/G Baseline | 1.44 (1.01–2.06) | 0.044* | 1.00 (0.68–1.46) | 0.994 |
|           | Recessive | G/G – G/A Baseline | 0.75 (0.34–1.64) | 0.470 | 0.67 (0.26–1.76) | 0.420 |
|           | Log–additive | – | 1.23 (0.92–1.65) | 0.165 | 0.94 (0.68–1.30) | 0.712 |

Abbreviations: CI, confidence interval; OR, odds ratio; SNP: single nucleotide polymorphism.

*p* < 0.05 indicates statistical significance.

#### FIGURE 1
Haplotype block map for SNPs in FOXO3. The numbers inside the diamonds indicate the D’ for pairwise analyses.
Examination of haplotype frequencies and LD patterns was also important for identification of the appropriated populations for tag SNP selection (Gabriel et al., 2002). Our results suggested that rs12212067, rs3800229 and rs4946935 compose an LD block (all pairwise D’ > 0.80). The results did not reveal a significant correlation between common haplotype and TB risk (all p > 0.05).

Furthermore, Regulome DB (http://www.regulomedb.org/) and the prediction tools SNPinfo (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) were performed to determine the potential functional effect of the SNPs. No data were provided for the functional prediction of rs3800229 and rs4946935. Hence, further molecular characterization is need to explore in vivo and in vitro experiments.

There were several limitations in our study. First, in the case–control study, the selection bias could not be avoided due to the hospital-based design. Second, the information of our samples were incomplete, the association between clinical parameters and TB risk were need to explore. In the future, we would like to collect clinical characteristics to evaluate this difference between the case and control group.

In conclusion, our result is the first to confirm that rs3800229 and rs4946935 of \( \text{FOXO3} \) were associated with increased risk of TB in a Han Chinese population. In further studies, the causal variant(s) and the molecular mechanisms underlying the association with TB need to be researched.

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**CONFLICTS OF INTEREST**

The authors have declared that they have no conflict of interest.

**ETHICS APPROVAL**

All participants were informed in writing and verbally of the procedures, and purpose of this study and signed informed consent documents were obtained from both patients and controls. Study protocols were approved by the ethics committee of the Xi’an Chest Hospital, and complied with the ethical standards of the Ethical Committee and World Medical Association Declaration of Helsinki. All research analyses were carried out in accordance with the approved guidelines and regulations.

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**REFERENCES**

Bharti, D., Kumar, A., Mahla, R. S., Kumar, S., Ingle, H., Shankar, H., … Kumar, H. (2014). The role of TLR9 polymorphism in susceptibility to pulmonary tuberculosis. *Immunogenetics, 66*(12), 675–681. https://doi.org/10.1007/s00251-014-0806-1

Chen, C., Liu, Q., Zhu, L., Yang, H., & Lu, W. (2013). Vitamin D receptor gene polymorphisms on the risk of tuberculosis, a meta-analysis of 29 case-control studies. *PLoS ONE, 8*(12), e83843. https://doi.org/10.1371/journal.pone.0083843

Dejean, A. S., Beisner, D. R., Ch'en, I. L., Kerdiles, Y. M., Babour, A., Arden, K. C., … Hedrick, S. M. (2009). Transcription factor Foxo3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. *Nature Immunology, 10*(5), 504–513. https://doi.org/10.1038/ni.1729

Du, B., Hua, D., Droma, C., Zhang, H., Qu, J., Yang, L., … Yang, J. (2017). Association between TAP2 and SEC14L2 polymorphisms and pulmonary tuberculosis risk in the Tibetan Chinese population. *International Journal of Clinical and Experimental Pathology, 10*(11), 11188–11194.

Flachsbart, F., Dose, J., Gentschew, L., Geismann, C., Caliebe, A., Knecht, C.,… Nebel, A. (2017). Identification and characterization of two functional variants in the human longevity gene FOXO3. *Nature Communications, 8*(1), https://doi.org/10.1038/s41467-017-02183-y

Gabriel, S. B., Schaffner, S. F., Nguyen, H., Moore, J. M., Roy, J., Blumenstiel, B., … Altshuler, D. (2002). The structure of haplotype blocks in the human genome. *Science, 296*(5576), 2225–2229. https://doi.org/10.1126/science.1069424

Greer, E. L., & Brunet, A. (2008). FOXO transcription factors in ageing and cancer. *Acta Psychologica, 192*(1), 19–28. https://doi.org/10.1111/j.1748-1716.2007.01780.x

**TABLE 6** Haplotype analysis of \( \text{FOXO3} \) SNPs in association with the risk of tuberculosis

| Haplotype | Freq | Crude analysis | Adjusted by gender and age |
|-----------|------|---------------|---------------------------|
|           | TB   | Controls      | OR (95% CI)               | OR (95% CI)               |
|           | p-Value | p           | p                         | p                          |
| GGA       | 0.13 | 0.12          | 0.524                     | 1.09 (0.84–1.41)           | 1.09 (0.84–1.42) |
|           |      |               |                           |                           | 0.508                   |
| TGA       | 0.89 | 0.90          | 0.538                     | 0.92 (0.69–1.21)           | 0.92 (0.69–1.22) |
|           |      |               |                           |                           | 0.545                   |
| TTG       | 0.76 | 0.78          | 0.343                     | 0.90 (0.73–1.11)           | 0.90 (0.73–1.11) |
|           |      |               |                           |                           | 0.329                   |

**Note:** Block comprised of the three closely linked SNPs rs12212067, rs3800229 and rs4946935. p values were calculated with \( \chi^2 \) tests.

Abbreviations: CI, confidence interval; OR, odds ratio.

\( p < 0.05 \) indicates statistical significance.
Haoues, M., Refai, A., Mallavialle, A., Barbouche, M. R., Laabidi, N., Deckert, M., & Essafi, M. (2014). Forkhead box O3 (FOXO3) transcription factor mediates apoptosis in BCG-infected macrophages. *Cellular Microbiology, 16*(9), 1378–1390. https://doi.org/10.1111/cmi.12298

Hedrick, S. M., Hess Michelini, R., Doedens, A. L., Goldrath, A. W., & Stone, E. L. (2012). FOXO transcription factors throughout T cell biology. *Nature Reviews Immunology, 12*(9), 649–661. https://doi.org/10.1038/nri3278

Hosaka, T., Biggs, W. H. 3rd, Tieu, D., Boyer, A. D., Varki, N. M., Hedrick, S. M., Hess Michelini, R., Doedens, A. L., Goldrath, A. W., & Marlow, G., Han, D. Y., Triggs, C. M., & Ferguson, L. R. (2015). Food Maiese, K., Chong, Z. Z., Hou, J., & Shang, Y. C. (2009). The “O” class: FOXO transcription factor (FOXO) family members in mice reveals their transcriptional outcomes in inflammatory and infectious disease to a FOXO3-regulated pathway. *Cell, 155*(1), 57–69. https://doi.org/10.1016/j.cell.2013.08.034

Junqueira, C., Caetano, B., Bartholomeu, D. C., Melo, M. B., Ropert, C., Rodrigues, M. M., & Gazzinelli, R. T. (2010). The endless race between Trypanosoma cruzi and host immunity: Lessons for and beyond Chagas disease. *Expert Reviews in Molecular Medicine, 12*, e29. https://doi.org/10.1017/s1462399410001560

Langhorne, J., Albano, F. R., Hensmann, M., Sanni, L., Cadman, E., Voisine, C., & Sponaas, A. M. (2004). Dendritic cells, pro-inflammatory responses, and antigen presentation in a rodent malaria infection. *Inmunological Reviews, 201*, 35–47. https://doi.org/10.1111/j.0105-2896.2004.00182.x

Lee, J. C., Espéli, M., Anderson, C. A., Linterman, M. A., Pocock, J. M., Williams, N. J., … Barrett, J. C. (2013). Human SNP links differential outcomes in inflammatory and infectious disease to a FOXO3-regulated pathway. *Cell, 155*(1), 57–69. https://doi.org/10.1016/j.cell.2013.08.034

Liu, Q., Wang, J., Sandford, A. J., Wu, J., Wang, Y. U., Wu, S., … He, J.-Q. (2015). Association of CYBB polymorphisms with tuberculosis susceptibility in the Chinese Han population. *Infection, Genetics and Evolution, 33*, 169–175. https://doi.org/10.1016/j.meegid.2015.04.026

Lu, Y., Zhu, Y., Wang, X., Wang, F., Peng, J., Hou, H., & Sun, Z. (2016). FOXO3 rs12212067: T > G association with active tuberculosis in Han Chinese population. *Inflammation, 39*(1), 10–15. https://doi.org/10.1007/s10753-015-0217-y

Maiase, K., Chong, Z. Z., Hou, J., & Shang, Y. C. (2009). The “O” class: Crafting clinical care with FoxO transcription factors. *Advances in Experimental Medicine and Biology, 665*, 242–260. https://doi.org/10.1007/978-1-4419-1599-3_18

Marlow, G., Han, D. Y., Triggs, C. M., & Ferguson, L. R. (2015). Food intolerance: Associations with the rs12212067 polymorphism of FOXO3 in Crohn’s disease patients in New Zealand. *Journal of Nutrigenetics and Nutrigenomics, 8*(2), 70–80. https://doi.org/10.1159/000435783

Miao, R., Ge, H., Xu, L., & Xu, F. (2014). CD14-159C/T polymorphism contributes to the susceptibility to tuberculosis: Evidence from pooled 1,700 cases and 1,816 controls. *Molecular Biology Reports, 41*(5), 3481–3486. https://doi.org/10.1007/s11033-014-3210-x

Murray, M., Oxlade, O., & Lin, H. H. (2011). Modeling social, environmental and biological determinants of tuberculosis. *The International Journal of Tuberculosis and Lung Disease, 15*(Suppl 2), 64–70. https://doi.org/10.5588/ijtld.10.0535

Nguetse, C. N., Croinesser, P. G., & Velavan, T. P. (2015). FOXO3A regulatory polymorphism and susceptibility to severe malaria in Gabonese children. *Immunogenetics, 67*(2), 67–71. https://doi.org/10.1007/s00251-014-0816-z

Qraflı, M., Amay, Y., Bourkadi, J., Amor, J. B., Iraki, G., Bakri, Y., … Sadki, K. (2014). The CYP7A1 gene rs3808607 variant is associated with susceptibility of tuberculosis in Moroccan population. *Pan African Medical Journal, 18*, 1. https://doi.org/10.11604/pamj.2014.18.1.3397

Rodriguez-Castillo, J. A., Arce-Mendoza, A. Y., Quintanilla-Siller, A., Rendon, A., Salinas-Carmona, M. C., & Rosas-Taraco, A. G. (2015). Possible association of rare polymorphism in the ABCB1 gene with rifampin and ethambutol drug-resistant tuberculosis. *Tuberculosis (Edinb), 95*(5), 532–537. https://doi.org/10.1016/j.tube.2015.04.004

Sun, L., Hu, C., Zheng, C., Qian, Y. U., Liang, Q., Lv, Z., … Yang, Z. E. (2015). FOXO3 variants are beneficial for longevity in Southern Chinese living in the Red River Basin: A case-control study and meta-analysis. *Scientific Reports, 5*, 9852. https://doi.org/10.1038/srep09852

Thomsen, V. Ø., Andersen, A. B., Zhang, X., Mørrø, C. F., Yang, Z., Kamper-Jørgensen, Z., … Zhang, L. (2011). Effect of sex, age, and race on the clinical presentation of tuberculosis: A 15-year population-based study. *American Journal of Tropical Medicine and Hygiene, 85*(2), 285–290. https://doi.org/10.4269/ajtmh.2011.10-0630

Wang, J., & Shen, H. (2009). Review of cigarette smoking and tuberculosis in China: Intervention is needed for smoking cessation among tuberculosis patients. *BMC Public Health, 9*, 292. https://doi.org/10.1186/1471-2458-9-292

Watkins, S. K., & Hurwitz, A. A. (2012). FOXO3: A master switch for regulating tolerance and immunity in dendritic cells. *Oncoimmunology, 1*(2), 252–254. https://doi.org/10.4269/ajtmh.2011.10-0630

Zhu, L., Liu, L., He, X., Yan, M., Du, J., Yang, H., … Jin, T. (2017). Association between genetic polymorphism of telomere-associated gene ACYP2 and the risk of HAPE among the Chinese Han population: A case-control study. *Medicine (Baltimore), 96*(13), e6504. https://doi.org/10.1097/md.0000000000006504

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