Associations of Genetic Variants of miRNA Coding Regions With Pulmonary Tuberculosis Risk in China

Mingwu Zhang
  Zhejiang Provincial Center for Disease Control and Prevention

Zhengwei Liu
  Zhejiang Provincial CDC: Zhejiang Provincial Center for Disease Control and Prevention

Yelei Zhu
  Zhejiang Provincial Center for Disease Control and Prevention

SongHua Chen
  Zhejiang Provincial Center for Disease Control and Prevention

Bin Chen
  Zhejiang Provincial Center for Disease Control and Prevention

Xiaomeng Wang (✉ 417066781@qq.com)
  Zhejiang Provincial Center for Disease Control and Prevention, Binsheng Road, Binjiang District, Hangzhou City,
Zhejiang Province, China

Research article

Abstract

Background Tuberculosis (TB) is the leading cause of death caused by single pathogenic microorganism of mycobacterium tuberculosis(MTB). The study aims to explore the associations of microRNA (miRNA) single nucleotide polymorphisms (SNPs) with pulmonary TB (PTB) risk.

Methods A population-based case-control study was conducted, and 168 newly diagnosed smear-positive PTB cases and 251 non-TB controls were recruited. SNPs located within miR-27a (rs895819), miR-423 (rs6505162), miR-196a-2 (rs11614913), miR-146a (rs2910164), miR-618 (rs2682818) were selected and MassARRAY® MALDI-TOF System was employed for genotyping. SPSS19.0 was adopted for statistical analysis, non-conditional logistic regression was performed. Odds ratios (ORs) and 95%confidence intervals (95%CIs) were computed to estimate the associations. Associations of haplotypes with PTB risk was performed with online tool.

Results Rs895819 CT/CC genotype was associated with reduced PTB risk among female population (OR= 0.45, 95%CI: 0.23-0.98), p=0.045. Haplotypes (Combined with rs895819, rs2682818, rs2910164, rs6505162 and rs11614913) TCCCT, TAGCC, CCCCC, CCGCT and TCGAT were associated with reduced PTB risk and the ORs were 0.67 (95%CI: 0.45-0.99), 0.49 (0.25-0.94), 0.34 (95%CI: 0.14-0.81), 0.22 (95%CI: 0.06-0.84) and 0.24 (95%CI: 0.07-0.79)⬚ respectively; while the haplotypes of TAGCT, CCCCT, CACCT and TCCAT were associated with increased PTB risk, and the ORs were 3.63 (95%CI: 1.54-8.55), 2.20 (95%CI: 1.00-4.86), 3.90 (95%CI: 1.47-10.36) and 2.95 (95%CI: 1.09-7.99), respectively.

Conclusions Rs895819 CT/CC genotype was associated with reduced female PTB risk and haplotype TCCCT, TAGCC, CCCCC, CCGCT and TCGAT were associated with reduced PTB risk, while TAGCT, CCCCT, CACCT and TCCAT were associated with increased risk.

Background

In 2018 about 10.0 million people fell ill with tuberculosis (TB), while 1.2 million HIV-negative individuals and an additional 251000 HIV-positive individuals died of TB worldwide (Organization, 2019). Although that year an estimated 1.7 billion people harbored *Mycobacterium tuberculosis* (MTB) infections, only a relatively small proportion (5–10%) of them would potentially develop active TB during their lifetimes.

The progression from MTB infection to TB disease is influenced by multiple factors, including undernutrition, smoking, alcohol consumption, etc. (Abedi et al., 2019). In addition, host molecular regulatory mechanisms, such as gene expression regulation by microRNAs (miRNAs), may also be involved in disease progression. MiRNAs are a class of non-coding RNAs that play key roles in regulating post-transcriptional gene expression during numerous physiological and pathological processes, including MTB infection and TB progression to active disease. During MTB infection, which lies at the root of active TB disease in humans, downregulated expression of microRNAs homo sapiens (hsa)-miR-29a and hsa-miR-15b has been reported. By contrast, hsa-miR-576-5p, hsa-miR-500 and hsa-miR-155 expression have been shown to be upregulated during latent tuberculosis infection (LTBI) (Lu et al., 2019). Intriguingly, miRNA effects have been demonstrated during different stages of TB progression; for example, Beibei Fu et al. found that miR-325-3p was upregulated after MTB infection then demonstrated that miR-325-deficient mice exhibited MTB resistance. These findings collectively shed light on the immune escape pathway used by MTB, whereby MTB infection directly targeted LNX1 expression that led to anti-apoptotic STAT3 signaling that ultimately promoted intracellular MTB survival (Fu et al., 2020). Meanwhile, another study showed that hsa-miR-29a-3p, hsa-miR-155-5p and hsa-miR-361-5p were upregulated during active tuberculosis (ATB) as compared to their expression in healthy subjects; of these miRNAs, hsa-miR-29a-3p, and hsa-miR-361-5p were found to be upregulated during ATB as

compared to their expression during LTBI (Ndzi et al., 2019). Notably, MiR-143 and miR-365 have been pinpointed as key regulators of MTB infection, with roles in MTB-infected macrophages that influence regulation of host cell c-Maf, Bach-1 and Elmo-1 activities (Tamgue et al., 2019). In another study, a mimic of miR-708-5p enhanced intracellular mycobacterial survival during MTB infection, while miR-708-5p downregulation suppressed MTB survival (Li and Zhang, 2019). Meanwhile, another study found that upregulation of miR-579 induced macrophage cytotoxicity that countered MTB infection by targeting the cPWWP2A-miR-579 axis to ultimately protect human macrophages from MTB infection (Ma et al., 2019).

Genetic variants of expressed miRNA regions have been implicated in their biogenic effects (Kroliczewski et al., 2018); these effects appear to influence or alter downstream biological processes to cause disease (Jazdzewski and de la Chapelle, 2009). For example, the miR-146a single nucleotide polymorphism (SNP) rs2910164 has been shown to be associated with ischemic stroke incidence and prognosis (Mullany et al., 2015; Qu et al., 2016; Wang et al., 2020). Meanwhile, other studies have revealed that miRNAs participate in MTB infection and TB incidence, although studies showing relationships between miRNA SNPs and TB incidence risk have been limited or have remained obscure. In the present study, selected SNPs within expressed sequences of miR-27a (rs895819), miR-423 (rs6505162), miR-196a-2 (rs11614913), miR-146a (rs2910164) and miR-618 (rs2682818) were analyzed for significant associations with TB incidence.

Methods

2.1 Ethics statement

The study was approved by the ethics committee of the Zhejiang Provincial Center for Disease Control and Prevention. Written informed consent was obtained from each participant prior to participation in the study.

2.2 Study population

A population-based case-control study was conducted in Jiangshan and Changshan counties in Zhejiang Province. In the controlled study, 168 newly diagnosed smear-positive pulmonary TB (PTB) patients were enrolled from January 1st, 2016 to June 30th, 2017 along with 251 control subjects recruited from the same hospitals. Control subjects included clinically diagnosed patients suffering from non-TB illnesses or healthy individuals. All recruited controls verified they had no history of TB.

All participants were asked to complete a structured questionnaire and provide personal demographic information and lifestyle-related characteristics, such as smoking, drinking, etc. Other information collected from each study subject included health-related factors and disease diagnosis and treatment histories.

2.3 Genotyping

In the present study, a MassARRAY® MALDI-TOF System (US, Sequenom, Inc.) was employed for genotyping. During genotyping, three primers for each SNP were designed and primer sequences are shown in Table 1. The genotyping protocol was implemented as described below.

(1) Polymerase chain reaction (PCR)

Each PCR amplification reaction was prepared in a total volume of 5 µL and contained 1 µL (30 ng) DNA template, 0.625 µL of 10× PCR buffer (15 mM $MgCl_2$, Qiagen, Germany), 0.325 µL of $MgCl_2$ (25 mM, Qiagen, Germany), 1 µL of dNTPs (2.5 mM each, TaKaRa, Dalian, China), 0.1 µL of Hotstar Taq (0.5 U, Qiagen, Germany), 1 µL of diluted each

primers and 0.95 μL of ddH$_2$O. PCR cycling parameters were as follows: DNA denaturation at 94 °C for 10 min followed by 35 cycles of 94 °C for 20 s, 56 °C for 20 s, and 72 °C for 60 s followed by a final extension step of 72 °C for 3 min and storage at 4 °C until needed.

(2) SPA typing reaction preparation

For SPA typing, 2 μL of SPA reaction buffer was added into each PCR plate well then each reaction was incubated using the following parameters: 37 °C for 40 min followed by 85 °C for 5 min. Completed reactions were maintained at 4 °C until needed.

(3) Single base extension

For single base extension, 2 μL of iPlex reaction reagent (supplied by SEQUENOM as a solution containing 0.2 μL of 10× iPlex Buffer, 0.2 μL of iPlex Termination Mix, 0.804 μL of diluted primer and 0.041 μL of iPlex enzyme). PCR reaction parameters was set as follows: one cycle at 94 °C for 30 s followed by 40 cycles of annealing at 94 °C for 5 s then 5 cycles of 52 °C for 5 s and 80 °C for 5 s then a final extension step at 72 °C for 3 min. Completed reactions were stored at 4 °C until needed.

(4) Reaction product purification and data analysis

Reaction products were purified and spotted onto sample plates according to standard protocols. Draft data were generated then processed using TYPER genotyping analysis software.

10% of randomly selected DNA samples were rechecked using the same method.

2.4 Statistical analysis

In the present study, SPSS software version 19.0 was used for statistical analysis. Chi-square tests were used to compare distributions of demographic factors, including gender, occupation, family income, nationality, smoking and alcohol use. Means ages of PTB patient and control groups were compared using independent-t tests. Non-conditional logistic regression was employed, with inclusion of age, sex, smoking and alcohol use in regression models as covariates followed by computing of adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs). Haplotype analyses for all five SNPs were performed using online software (http://analysis.bio-x.cn/myAnalysis.php); haplotypes with frequencies less than 0.03 were excluded from the final analysis. ORs and 95% CIs for each tested haplotype were compared to all other haplotypes in order to estimate the contribution of each haplotype to PTB risk. A two-sided p-value less than 0.05 was considered significant.

Results

3.1 Distributions of demographic characteristics

Comparisons of demographic characteristics between groups of PTB cases and controls were performed and are shown in Table 2. Participants in the control group were younger than those in the case group (t = -2.602, p = 0.010). A higher proportion of alcohol users was found in the control group as compared to the case group (37.8% *vs.* 24.2%), p = 0.005. The proportion of subjects with annual household income per capita ≥ 10000 RMB in the control group was higher than the proportion in the case group (67.5% *vs.* 54.2%, respectively), p = 0.014. No significant intergroup differences were detected for sex, occupation, smoking and nationality.

3.2 Association of miRNA SNPs with PTB risk

From Table 3, it can be seen that none of the SNPs selected for study, including miR-27a (rs895819), miR-423 (rs6505162), miR-196a-2 (rs11614913), miR-146a (rs2910164) and miR-618 (rs2682818), were associated with PTB risk in the overall study population. However, after stratification by sex, associations of male and female populations with PTB incidence were determined and are shown in Table 4. A significant association was detected between the rs895819CT/CC genotype and TB risk for the female population, as reflected by OR = 0.45 (95%CI: 0.23-0.98) (Table 4), while no significant association was detected between this genotype and PTB incidence for the male population. Meanwhile, no significant associations were observed between other SNPs and PTB risk for either the male or female population.

After stratification by age, associations between PTB risk and genotype for those aged < 60 years and ≥ 60 were determined and are shown in Table 5. No significant associations between PTB risk and genotype were detected among those aged < 60 years. Conversely, in those aged ≥ 60 years (Table 5), a significant association was detected between the rs895819CT genotype and increased PTB risk (OR = 0.53, 95%CI: 0.28-0.99), although no significant association was found between genotype rs895819CT/CC and reduced PTB risk (OR = 0.59, 95%CI: 0.32-1.06). However, a marginally significant association was found between the rs6505162AA/AC genotype and increased PTB risk for those aged ≥ 60 years (OR = 1.69, 95%CI: 0.93-3.06), p = 0.083.

3.3 Associations of haplotypes with PTB risk

Haplotypes with frequencies less than 0.03 were excluded from the final analysis. Associations between PTB risk and the remaining haplotypes of SNPs rs895819, rs2682818, rs2910164, rs6505162 and rs11614913 are summarized in Table 6.

Of all included haplotypes, TCCCT had the highest frequency and was significantly associated with reduced PTB risk (OR = 0.67, 95%CI: 0.45-0.99), p = 0.047. Among the other haplotypes, similar significant associations were detected for TAGCC, CCCCC, CCGCT and TCGAT, with ORs of 0.49 (95%CI: 0.25-0.94), 0.34 (95%CI: 0.14-0.81), 0.22 (95%CI: 0.06-0.84) and 0.24 (95%CI: 0.07-0.79), respectively. By contrast, haplotypes TAGCT, CCCCT, CACCT and TCCAT were associated with increased TB risk, with ORs of 3.63 (1.54-8.55), 2.20 (1.00-4.86), 3.90 (1.47-10.36) and 2.95 (1.09-7.99), respectively.

Discussion

In this study, SNPs located within expressed miRNA regions and their associations with PTB risk were analyzed. After stratification of results according to sex, we observed a significant association between rs895819 and overall TB risk of the female population. Several haplotypes of five SNPs were found to be significantly associated with PTB incidence.

MiRNAs, a class of micro-molecules, play key roles in gene expression regulation and in multiple biological and pathological processes, including embryonic development, tumorigenesis, immunoregulation and inflammation (Dai and Ahmed, 2011). When considered together, SNPs located within expressed miRNA regions and binding sites may be useful for predicting an individual's future risk of contracting infectious and non-infectious diseases. To date, SNPs and miRNA sequence-based variations have been applied to the prediction of tumor-associated diseases, asthma, TB, cardiovascular and cerebrovascular diseases (among others) in recent decades (Chen et al., 2018b; Smits et al., 2011). For example, the SNP located within the 3' untranslated region (3' UTR) of the phosphatase and tensin homolog (*PTEN*) gene has been found to be associated with cervical cancer risk (Yu et al., 2020). Another miRNA, miR-27a, has also been found to play key roles in regulating gene expression and proliferation of ovarian cancer and breast cancer cells (Ljepoja et al., 2019; Zhang et al., 2019). In the present study, one SNP located within the expressed sequence of

miR-27a was found to be associated with decreased PTB incidence in the female population, but not in the male population. Intriguingly, Wang et al. found that downregulation of miR-27a expression in MTB-infected THP-1 cells led to enhanced expression of interferon-γ (IFN-γ), interleukin-beta (IL-β), IL-6 and tumor necrosis factor-alpha (TNF-α); this enhanced cytokine expression was abolished by cell transfection with miR-27a mimics that also led to downregulated target gene IRAK4 expression (Wang et al., 2017). Taken together, these findings offer clues that will likely enhance our understanding of potential molecular mechanisms associated with TB disease incidence. Indeed, progress has already been made toward this goal in another study that verified that ER-located Ca(2+) transporter CACNA2D3 was another miR-27a target gene. When miR-27a activity was suppressed, downregulation of Ca(2+) signaling and subsequent inhibition of autophagosome formation occurred that promoted greater intracellular MTB survival (Liu et al., 2018). Meanwhile, another SNP located within the expressed region of miR-27a was found to be associated with increased colorectal cancer (CRC) risk and reduced breast cancer risk (Mashayekhi et al., 2018; Zhang et al., 2013; Zhang et al., 2020). These studies indicate that SNPs located within the expressed region of miR-27a were functional SNPs with putative roles in gene regulation that consequently influenced the occurrences of certain diseases.

Other miRNAs have been linked to human diseases as well. For example, results of one study implicated involvement of both miR-146 and miR-29 in post-transcriptional regulation of the renalase gene, a gene which contributes to observed inter-individual variations of cardiometabolic traits (Kalyani et al., 2015). In other studies, the SNP located within miR-146a (rs2910164) was confirmed to be associated with increased coronary artery disease (CAD) risk (Xiong et al., 2014) and reduced gastric cancer risk (Jiang et al., 2016). Although these studies also indicated that the SNP located within miR-146a was a functional SNP, this SNP did not appear to be associated with PTB incidence in the current study and thus was not found to affect TB risk. Similarly, other studies have shown that SNPs located within miR-196a-2 were associated with oral squamous cell carcinoma (OSCC) survival (Liu et al., 2013), but not with breast cancer risk (Mashayekhi et al., 2018) or colorectal cancer (CRC) risk (Hezova et al., 2012), aligning with results of this study showing no association of miR-196a-2 with PTB risk.

Another miRNA linked to huma disease, miR-423, was shown in one study to activate NF-κβ and promote breast cancer invasion (Dai et al., 2020) while miR-423-5p was shown in another study to be upregulated during active TB, highlighting its potential as a PTB biomarker (Tu et al., 2019). Meanwhile, the SNP (rs6505162) located within the expressed miR-423 region was associated with lower risks of gastrointestinal cancer, CRC and lung cancer, but was not associated with higher risks of esophageal cancer, breast cancer or gastric cancer (Moazeni-Roodi et al., 2019). Moreover, previous studies had identified miR-423 as a key factor associated with TB incidence that exerted its functional effects via gene regulation, although here this SNP was not found to be significantly associated with PTB incidence.

Yet another miRNA liked to human disease, miR-618, has been reported to suppress gastric cancer metastasis by downregulating transforming-growth factor beta 2 (TGF-2β) expression (Shi et al., 2019), and to inhibit prostate cancer cell migration and invasion by targeting FOXP2 and downregulating TGF-β (Song et al., 2017). Meanwhile, results of a meta-analysis study revealed that SNP rs2682818, located within the expressed sequence region of miR-618, was not associated with overall cancer risk, but was specifically associated with breast cancer risk (Feng et al., 2019). However, in another study rs2682818 was linked to reduced CRC risk (Chen et al., 2018a).

Haplotype analysis is a method that evaluates various combinations of multiple genetic variants to find haplotypes that are useful for predicting disease risk. In the present study, combinations of various haplotypes were evaluated to determine their associations with PTB risk. Ultimately, a strategy for predicting future disease incidence that was based on multiple SNPs was found to be superior to one based on a single SNP.

In order to discover potential SNP-disease associations for different age groups, stratification by age was conducted. Notably, SNPs with marginal levels of statistical significance were detected within expressed sequences of miR-423 and miR-27a for subjects aged ≥ 60 years, but not for subjects aged < 60 years. Thus, we speculate that significant SNP correlations with PTB disease may were not detected for some age groups, due to their statistically inadequate sample sizes. As a result, although the present study provided some useful information, further studies based on larger sample sizes are needed to verify the conclusions of this study and to discover SNPs with statistically stronger correlations with PTB risk.

Conclusions

Based on the results of this study, we conclude that rs895819 was associated with reduced female PTB incidence, while this SNP together with rs6505162 may be useful for predicting PTB risk in patients aged ≥ 60 years of age. Analysis of haplotypes with regard to SNPs rs895819, rs2682818, rs2910164, rs6505162 and rs11614913 may find useful markers for effective prediction of PTB risk.

Abbreviations

PTB: pulmonary tuberculosis; MTB: mycobacterium tuberculosis, miRNAs: microRNAs; SNP: single nucleotide polymorphism; LTBI: latent tuberculosis infection; ATB: active tuberculosis; TB: tuberculosis; PCR: Polymerase chain reaction; ORs: odds ratios; 95% CIs: 95% confidence intervals; 3' UTR: 3' untranslated region; PTEN: phosphatase and tensin homolog; IFN-γ: interferon-γ; IL-β: interleukin-beta; TNF-α: tumor necrosis factor-alpha; OSCC: oral squamous cell carcinoma; CRC: corolectal cancer; TGF-beta: transforming growth factor-beta;

Declarations

References

1. Abedi, S., Moosazadeh, M., Tabrizi, R., Afshari, M., Nezammahalleh, A., Akbari, M., 2019. The impact of diabetics and smoking on gender differences of smear positive pulmonary tuberculosis incidence. The Indian journal of tuberculosis 66, 353-357.

2. Chen, Y., Du, M., Chen, W., Zhu, L., Wu, C., Zhang, Z., Wang, M., Chu, H., Gu, D., Chen, J., 2018a. Polymorphism rs2682818 in miR-618 is associated with colorectal cancer susceptibility in a Han Chinese population. Cancer medicine 7, 1194-1200.

3. Chen, Y., Luo, Z., Zeng, W., Lai, F.Y., Chen, Q.H., Lu, Z., Song, Y.Y., 2018b. [Associations of the MicroRNA-143/145 Polymorphisms with Cardiovascular Risk Factors and the Severity of Coronary Heart Disease]. Zhongguo yi xue ke xue yuan xue bao. Acta Academiae Medicinae Sinicae 40, 510-518.

4. Dai, R., Ahmed, S.A., 2011. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Translational research : the journal of laboratory and clinical medicine 157, 163-179.

5. Dai, T., Zhao, X., Li, Y., Yu, L., Li, Y., Zhou, X., Gong, Q., 2020. miR-423 Promotes Breast Cancer Invasion by Activating NF-kappaB Signaling. OncoTargets and therapy 13, 5467-5478.

6. Feng, X., Ji, D., Liang, C., Fan, S., 2019. Does miR-618 rs2682818 variant affect cancer susceptibility? Evidence from 10 case-control studies. Bioscience reports 39.

7. Fu, B., Xue, W., Zhang, H., Zhang, R., Feldman, K., Zhao, Q., Zhang, S., Shi, L., Pavani, K.C., Nian, W., Lin, X., Wu, H., 2020. MicroRNA-325-3p Facilitates Immune Escape of Mycobacterium tuberculosis through Targeting LNX1 via NEK6 Accumulation to Promote Anti-Apoptotic STAT3 Signaling. mBio 11.

8. Hezova, R., Kovarikova, A., Bienertova-Vasku, J., Sachlova, M., Redova, M., Vasku, A., Svoboda, M., Radova, L., Kiss, I., Vyzula, R., Slaby, O., 2012. Evaluation of SNPs in miR-196-a2, miR-27a and miR-146a as risk factors of colorectal cancer. World journal of gastroenterology 18, 2827-2831.

9. Jazdzewski, K., de la Chapelle, A., 2009. Genomic sequence matters: a SNP in microRNA-146a can turn anti-apoptotic. Cell cycle 8, 1642-1643.

10. Jiang, J., Jia, Z.F., Cao, D.H., Wu, Y.H., Sun, Z.W., Cao, X.Y., 2016. Association of the miR-146a rs2910164 polymorphism with gastric cancer susceptibility and prognosis. Future oncology 12, 2215-2226.

11. Kalyani, A., Sonawane, P.J., Khan, A.A., Subramanian, L., Ehret, G.B., Mullasari, A.S., Mahapatra, N.R., 2015. Post-Transcriptional Regulation of Renalase Gene by miR-29 and miR-146 MicroRNAs: Implications for Cardiometabolic Disorders. Journal of molecular biology 427, 2629-2646.

12. Kroliczewski, J., Sobolewska, A., Lejnowski, D., Collawn, J.F., Bartoszewski, R., 2018. microRNA single polynucleotide polymorphism influences on microRNA biogenesis and mRNA target specificity. Gene 640, 66-72.

13. Li, W.T., Zhang, Q., 2019. MicroRNA-708-5p regulates mycobacterial vitality and the secretion of inflammatory factors in Mycobacterium tuberculosis-infected macrophages by targeting TLR4. European review for medical and pharmacological sciences 23, 8028-8038.

14. Liu, C.J., Tsai, M.M., Tu, H.F., Lui, M.T., Cheng, H.W., Lin, S.C., 2013. miR-196a overexpression and miR-196a2 gene polymorphism are prognostic predictors of oral carcinomas. Annals of surgical oncology 20 Suppl 3, S406-414.

15. Liu, F., Chen, J., Wang, P., Li, H., Zhou, Y., Liu, H., Liu, Z., Zheng, R., Wang, L., Yang, H., Cui, Z., Wang, F., Huang, X., Wang, J., Sha, W., Xiao, H., Ge, B., 2018. MicroRNA-27a controls the intracellular survival of Mycobacterium tuberculosis by regulating calcium-associated autophagy. Nature communications 9, 4295.

16. Ljepoja, B., Garcia-Roman, J., Sommer, A.K., Wagner, E., Roidl, A., 2019. MiRNA-27a sensitizes breast cancer cells to treatment with Selective Estrogen Receptor Modulators. Breast 43, 31-38.

17. Lu, Y., Wang, X., Dong, H., Wang, X., Yang, P., Han, L., Wang, Y., Zheng, Z., Zhang, W., Zhang, L., 2019. Bioinformatics analysis of microRNA expression between patients with and without latent tuberculosis infections. Experimental and therapeutic medicine 17, 3977-3988.

18. Ma, J., Chen, X.L., Sun, Q., 2019. microRNA-579 upregulation mediates death of human macrophages with mycobacterium tuberculosis infection. Biochemical and biophysical research communications 518, 219-226.

19. Mashayekhi, S., Saeidi Saedi, H., Salehi, Z., Soltanipour, S., Mirzajani, E., 2018. Effects of miR-27a, miR-196a2 and miR-146a polymorphisms on the risk of breast cancer. British journal of biomedical science 75, 76-81.

20. Moazeni-Roodi, A., Ghavami, S., Hashemi, M., 2019. Association Between miR-423 rs6505162 Polymorphism and Susceptibility to Cancer. Archives of medical research 50, 21-30.

21. Mullany, L.E., Wolff, R.K., Herrick, J.S., Buas, M.F., Slattery, M.L., 2015. SNP Regulation of microRNA Expression and Subsequent Colon Cancer Risk. PloS one 10, e0143894.

22. Ndzi, E.N., Nkenfou, C.N., Mekue, L.M., Zentilin, L., Tamgue, O., Pefura, E.W.Y., Kuiate, J.R., Giacca, M., Ndjolo, A., 2019. MicroRNA hsa-miR-29a-3p is a plasma biomarker for the differential diagnosis and monitoring of tuberculosis. Tuberculosis 114, 69-76.

23. Organization, W.H., 2019. Global tuberculosis report 2019.

24. Qu, J.Y., Xi, J., Zhang, Y.H., Zhang, C.N., Song, L., Song, Y., Hui, R.T., Chen, J.Z., 2016. Association of the MicroRNA-146a SNP rs2910164 with Ischemic Stroke Incidence and Prognosis in a Chinese Population. International journal of molecular sciences 17.

25. Shi, J., Gong, L., Chen, L., Luo, J., Song, G., Xu, J., Lv, Z., Tao, H., Xia, Y., Ye, Z., 2019. miR-618 Suppresses Metastasis in Gastric Cancer by Downregulating the Expression of TGF-beta2. Anatomical record 302, 931-940.

26. Smits, K.M., Paranjape, T., Nallur, S., Wouters, K.A., Weijenberg, M.P., Schouten, L.J., van den Brandt, P.A., Bosman, F.T., Weidhaas, J.B., van Engeland, M., 2011. A let-7 microRNA SNP in the KRAS 3'UTR is prognostic in early-stage colorectal cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 17, 7723-7731.

27. Song, X.L., Tang, Y., Lei, X.H., Zhao, S.C., Wu, Z.Q., 2017. miR-618 Inhibits Prostate Cancer Migration and Invasion by Targeting FOXP2. Journal of Cancer 8, 2501-2510.

28. Tamgue, O., Gcanga, L., Ozturk, M., Whitehead, L., Pillay, S., Jacobs, R., Roy, S., Schmeier, S., Davids, M., Medvedeva, Y.A., Dheda, K., Suzuki, H., Brombacher, F., Guler, R., 2019. Differential Targeting of c-Maf, Bach-1, and Elmo-1 by microRNA-143 and microRNA-365 Promotes the Intracellular Growth of Mycobacterium tuberculosis in Alternatively IL-4/IL-13 Activated Macrophages. Frontiers in immunology 10, 421.

29. Tu, H., Yang, S., Jiang, T., Wei, L., Shi, L., Liu, C., Wang, C., Huang, H., Hu, Y., Chen, Z., Chen, J., Li, Z., Li, J., 2019. Elevated pulmonary tuberculosis biomarker miR-423-5p plays critical role in the occurrence of active TB by inhibiting autophagosome-lysosome fusion. Emerging microbes & infections 8, 448-460.

30. Wang, J., Jia, Z., Wei, B., Zhou, Y., Niu, C., Bai, S., Tong, C., Song, J., Li, Y., 2017. MicroRNA-27a restrains the immune response to mycobacterium tuberculosis infection by targeting IRAK4, a promoter of the NF-kappaB pathway. International journal of clinical and experimental pathology 10, 9894-9901.

31. Wang, W., Cai, Y., Deng, G., Yang, Q., Tang, P., Wu, M., Yu, Z., Yang, F., Chen, J., Werz, O., Chen, X., 2020. Allelic-Specific Regulation of xCT Expression Increases Susceptibility to Tuberculosis by Modulating microRNA-mRNA Interactions. mSphere 5.

32. Xiong, X.D., Cho, M., Cai, X.P., Cheng, J., Jing, X., Cen, J.M., Liu, X., Yang, X.L., Suh, Y., 2014. A common variant in pre-miR-146 is associated with coronary artery disease risk and its mature miRNA expression. Mutation research 761, 15-20.

33. Yu, A., Zhang, J., Mei, Y., Zhong, H., Chen, S., Song, Q., 2020. Correlation Between Single Nucleotide Polymorphisms of an miRNA Binding Site in the 3'UTR of PTEN and Risk of Cervical Cancer Among the Han Chinese. Genetic testing and molecular biomarkers 24, 381-389.

34. Zhang, L.Y., Chen, Y., Jia, J., Zhu, X., He, Y., Wu, L.M., 2019. MiR-27a promotes EMT in ovarian cancer through active Wnt/-catenin signalling by targeting FOXO1. Cancer biomarkers : section A of Disease markers 24, 31-42.

35. Zhang, N., Huo, Q., Wang, X., Chen, X., Long, L., Jiang, L., Ma, T., Yang, Q., 2013. A genetic variant in pre-miR-27a is associated with a reduced breast cancer risk in younger Chinese population. Gene 529, 125-130.

36. Zhang, S., Han, Q., Zhu, K., Wang, Q., 2020. The association of miR-27a rs895819 polymorphism with colorectal cancer risk in Chinese population. Journal of clinical laboratory analysis, e23497.

Tables

Table1 Primers designed for the miRNA SNPs genotyping		
SNP *NO.*	*Primers	Sequences (5'-3')
rs895819	P1	ACGTTGGATGGGAACTTAGCCACTGTGAAC
	P2	ACGTTGGATGAGGGCTTAGCTGCTTGTGAG
	P3	CCCCCCTGTGAACACGACTTGG
rs6505162	P1	ACGTTGGATGTCCAAAAGCTCGGTCTGAGG
	P2	ACGTTGGATGACTGTCTCTCTTCACACTGC
	P3	TAGAAACTCAAGCGCGGG
rs11614913	P1	ACGTTGGATGCTGATCTGTGGCTTAGGTAG
	P2	ACGTTGGATGTCGACGAAAACCGACTGATG
	P3	CGGCAACAAGAAACTG
rs2910164	P1	ACGTTGGATGGAACTGAATTCCATGGGTTG
	P2	ACGTTGGATGCCACGATGACAGAGATATCC
	P3	GGTTTTGTCAGTGTCAGACCT
rs2682818	P1	ACGTTGGATGTTCCATGAGCTGCTGATGAA
	P2	ACGTTGGATGACGTACATGCAGTAGCTCAG
	P3	CAGGAGACAAGCAGG
*P1 and P2 were adopted for PCR amplification, and P3 for single base extension.		

Table2 Demographic characteristics of recruited populations				
Variables	Control (n, %)	Case (n, %)	t/χ^2	*p*-value
Age	53.4 ± 14.6	62.6 ± 17.5	-2.602	0.010
Sex			0.039	0.844
Male	162(64.5)	110(65.5)		
Female	89(35.5)	58(34.5)		
Occupation			1.858	0.173
Farmers	200(80.6)	123(75.0)		
Others	48(19.4)	41(25.0)		
Smoking			0.046	0.830
Yes	95(37.8)	58(38.9)		
No	156(62.2)	91(61.1)		
Drinking			7.828	0.005
Yes	94(37.8)	36(24.2)		
No	155(62.2)	113(75.8)		
Nationalities			-	0.567*
Chinese han	250(99.6)	166(98.8)		
Others	1(0.4)	2(1.2)		
Family income			5.985	0.014
≥10000 RMB	137(67.5)	71(54.2)		
ᐸ10000 RMB	66(32.5)	60(45.8)		
*Computed with fisher's exact test.				

Table3 Associations of the miRNA SNPs with PTB risk				
Variables	Control (n, %)	Case (n, %)	*Adjusted ORs(95%CIs)	*p*-value
rs895819 (miR-27a)				
TT	140(57.9)	113(67.7)	Reference	
CT	90(37.2)	48(28.7)	0.69(0.44-1.07)	0.095
CC	12(5.0)	6(3.6%)	0.67(0.24-1.88)	0.441
CT/CC	102(42.2)	54(32.3)	0.70(0.45-1.09)	0.110
rs6505162 (miR-423)				
CC	174(70.7)	110(65.5)	Reference	
CA	67(27.3)	50(29.7)	1.32(0.84-2.08)	0.228
AA	5(2.0)	8(4.8)	2.80(0.88-8.96)	0.082
AA/AC	72(29.3)	58(34.5)	1.37(0.87-2.14)	0.171
rs11614913 (miR-196a-2)				
TT	66(26.8)	52(31.0)	Reference	
CT	135(54.9)	77(45.8)	0.76(0.48-1.22)	0.256
CC	45(18.3)	39(23.2)	1.09(0.61-1.95)	0.768
CT/CC	180(73.2)	116(69.0)	0.81(0.51-1.28)	0.369
rs2910164 (miR-146a)				
CC	93(38.3)	61(36.7)	Reference	
CG	109(44.9)	86(51.8)	1.17(0.75-1.82)	0.488
GG	41(16.9)	19(11.5)	0.65(0.34-1.25)	0.199
CG/GG	150(61.7)	105(63.3)	0.98(0.63-1.51)	0.910
rs2682818 (miR-618)				
CC	117(48.3)	81(48.5)	Reference	
CA	107(44.2)	72(43.1)	0.96(0.63-1.47)	0.865
AA	18(7.4)	14(8.4)	1.04(0.48-2.23)	0.925
CA/AA	125(51.6)	86(51.5)	0.95(0.62-1.45)	0.810
*Adjusted for age, sex, smoking and drinking.				

Table 4 Associations of miRNA SNPs with PTB risk among male and female population								
Male					**Female**			
Variables	Control (n, %)	Case (n, %)	*Adjusted ORs(95%CIs)	*p*-value	Control (n, %)	Case (n, %)	*Adjusted ORs(95%CIs)	p-value
rs895819 (miR-27a)								
TT	92(58.6)	71(64.5)	Reference		48(56.5)	42(73.7)	Reference	
CT	57(36.4)	36(32.7)	0.96(0.54-1.69)	0.882	33(38.8)	12(21.1)	0.40(0.17-0.96)	0.039
CC	8(5.0)	3(2.8)	0.70(0.17-2.87)	0.615	4(4.7)	3(5.2)	0.79(0.17-3.82)	0.772
CT/CC	65(41.4)	39(35.5)	0.93(0.54-1.60)	0.785	37(43.5)	15(26.3)	0.45(0.23-0.98)	0.045
rs6505162 (miR-423)								
CC	108(68.4)	71(64.5)	Reference		66(75.0)	39(67.2)	Reference	
CA	47(29.7)	33(30.0)	1.22(0.69-2.17)	0.489	20(22.7)	17(29.3)	1.17(0.52-2.64)	0.700
AA	3(1.9)	6(5.5)	3.77(0.87-16.32)	0.076	2(2.3)	2(3.5)	1.71(0.23-12.85)	0.603
AA/AC	50(31.6)	39(35.5)	1.38(0.80-2.38)	0.253	22(25.0)	19(32.8)	1.22(0.56-2.66)	0.615
rs11614913 (miR-196a-2)								
TT	37(23.4)	34(30.9)	Reference		29(33.0)	18(31.0)	Reference	
CT	93(58.9)	47(42.7)	0.55(0.29-1.04)	0.067	42(47.7)	30(51.7)	1.05(0.48-2.32)	0.899
CC	28(17.7)	29(26.4)	1.23(0.58-2.60)	0.586	17(19.3)	10(17.3)	1.05(0.37-3.00)	0.926
CT/CC	121(76.6)	76(69.1)	0.71(0.39-1.28)	0.257	59(67.0)	40(69.0)	1.05(0.50-2.23)	0.894
rs2910164 (miR-146a)								
CC	60(38.2)	35(32.1)	Reference		33(38.4)	26(45.6)	Reference	
CG	73(46.5)	62(56.9)	1.23(0.69-2.18)	0.486	36(41.9)	24(42.1)	0.78(0.36-1.71)	0.536
GG	24(15.3)	12(11.0)	0.83(0.34-2.00)	0.671	17(19.7)	7(12.3)	0.57(0.20-1.68)	0.310
CG/GG	97(61.8)	74(67.9)	1.13(0.65-1.97)	0.655	53(61.6)	31(54.4)	0.72(0.35-1.49)	0.379
rs2682818 (miR-618)								
CC	76(48.4)	53(48.6)	Reference		41(48.2)	28(48.3)	Reference	

CA	70(44.6)	48(44.0)	0.94(0.54-1.64)	0.840	37(43.5)	24(41.4)	0.85(0.40-1.80)	0.669
AA	11(7.0)	8(7.4)	1.11(0.37-3.31)	0.848	7(8.3)	6(10.3)	1.08(0.29-3.95)	0.911
CA/AA	81(51.6)	56(51.4)	0.97(0.56-1.65)	0.896	44(51.8)	30(51.7)	0.88(0.43-1.80)	0.735
*Adjusted for age, smoking and drinking.								

Table 5 Associations of miRNA SNPs with PTB risk among different age groups								
Aged◻60years					Aged ≥ 60years			
Variables	Control (n, %)	Case (n, %)	*Adjusted ORs(95%CIs)	*p*-value	Control (n, %)	Case (n, %)	*Adjusted ORs(95%CIs)	p-value
rs895819 (miR-27a)								
TT	59(52.7)	34(57.6)	Reference		81(62.3)	78(72,9)	Reference	
CT	46(41.1)	24(40.7)	0.90(0.44-1.82)	0.760	44(33.9)	24(22.4)	0.53(0.28-0.99)	0.047
CC	7(6.2)	1(1.7)	0.28(0.03-2.49)	0.256	5(3.8)	5(4.7)	1.07(0.29-3.97)	0.925
CT/CC	53(47.3)	25(42.4)	0.82(0.41-1.63)	0.565	49(37.7)	29(27.1)	0.59(0.32-1.06)	0.075
rs6505162 (miR-423)								
CC	76(66.1)	39(65.0)	Reference		98(74.8)	70(65.4)	Reference	
CA	36(31.3)	18(30.0)	0.97(0.46-2.02)	0.930	31(23.7)	32(29.9)	1.53(0.83-2.83)	0.176
AA	3(2.6)	3(5.0)	1.78(0.33-9.59)	0.503	2(1.5)	5(4.7)	4.44(0.79-24.95)	0.090
AA/AC	39(33.9)	21(35.0)	1.04(0.52-2.10)	0.906	33(25.2)	37(34.6)	1.69(0.93-3.06)	0.083
rs11614913 (miR-196a-2)								
TT	32(27.8)	18(36.0)	Reference		34(26.0)	33(30.8)	Reference	
CT	67(58.3)	26(52.0)	0.72(0.32-1.62)	0.432	68(51.9)	51(47.4)	0.71(0.38-1.33)	0.283
CC	16(13.9)	16(32.0)	2.19(0.84-5.71)	0.109	29(22.1)	23(21.5)	0.72(0.34-1.57)	0.412
CT/CC	83(62.2)	42(84.0)	1.00(0.47-2.12)	0.999	97(74.0)	74(68.9)	0.71(0.39-)1.29	0.263
rs2910164 (miR-146a)								
CC	45(39.5)	21(36.2)	Reference		48(37.2)	39(36.4)	Reference	
CG	50(43.9)	31(53.4)	1.01(0.49-2.10)	0.978	59(45.7)	55(51.4)	1.16(0.64-2.11)	0.620
GG	19(16.6)	6(10.4)	0.72(0.24-2.11)	0.545	22(17.1)	13(12.2)	0.69(0.29-1.65)	0.403
CG/GG	69(60.5)	37(63.8)	0.93(0.47-1.85)	0.839	81(62.8)	68(63.6)	1.03(0.59-1.82)	0.914
rs2682818 (miR-618)								
CC	57(50.4)	32(54.2)	Reference		60(46.5)	48(44.9)	Reference	

CA	50(44.2)	24(40.7)	0.88(0.44-1.77)	0.724	57(44.2)	48(44.9)	0.94(0.53-1.67)	0.825
AA	6(5.4)	3(5.1)	1.19(0.27-5.25)	0.821	12(9.3)	11(10.2)	0.99(0.37-2.67)	0.985
CA/AA	56(49.6)	27(45.8)	0.91(0.46-1.79)	0.790	69(53.5)	59(55.1)	0.95(0.54-1.64)	0.843
*Adjusted for sex, smoking and drinking.								

Table 6 Associations of haplotypes with PTB risk						
*Haplotype	Case(n,%)	Control(n,%)	Chi^2	Fisher's p	Pearson's p	ORs (95%CI)
TCCCT	43(13.1)	85(17.8)	3.945	0.047	0.047	0.67 (0.45-0.99)
TCCCC	48(14.5)	59(12.4)	0.557	0.456	0.455	1.17 (0.77-1.77)
TCGCT	39(11.8)	45(9.5)	0.909	0.340	0.340	1.25 (0.79-1.97)
TCGCC	25(7.7)	21(4.4)	3.647	0.056	0.056	1.78 (0.98-3.25)
TAGCC	13(3.9)	35(7.4)	4.827	0.028	0.028	0.49 (0.25-0.94)
TACCT	17(5.0)	33(6.9)	1.438	0.230	0.230	0.69 (0.37-1.27)
TACCC	12(3.7)	18(3.8)	0.009	0.925	0.925	0.97 (0.46-2.03)
TAGCT	19(5.7)	8(1.6)	9.858	0.002	0.002	3.63 (1.54-8.55)
CCCCC	6(1.9)	26(5.4)	6.475	0.011	0.011	0.34 (0.14-0.81)
TCCAC	11(3.2)	19(4.0)	0.439	0.507	0.507	0.77 (0.36-1.67)
CCCCT	16(4.8)	11(2.2)	4.006	0.045	0.045	2.20 (1.00-4.86)
CACCT	15(4.6)	6(1.2)	8.551	0.003	0.003	3.90 (1.47-10.36)
TACAC	11(3.3)	7(1.6)	2.586	0.108	0.108	2.13 (0.83-5.45)
TCCAT	12(3.6)	6(1.2)	4.955	0.026	0.026	2.95 (1.09-7.99)
CCGCT	3(0.8)	16(3.3)	5.888	0.015	0.015	0.22 (0.06-0.84)
TCGAT	3(1.0)	18(3.9)	6.479	0.011	0.011	0.24 (0.07-0.79)
*Combined with SNPs of rs895819, rs2682818, rs2910164, rs6505162 and rs11614913 in turn.						

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Questionnaire.docx