Correlation analysis between SNPs in microRNA-machinery genes and tuberculosis susceptibility in the Chinese Uygur population

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Abstract
Tuberculosis (TB) is an infectious disease caused by a mycobacterial infection, with high morbidity and mortality worldwide. The pathogenesis of TB is still unclear; however, a growing body of evidence suggests that host genetic factors may play important roles in susceptibility to TB, and different gene polymorphisms in different ethnic and genetic backgrounds may lead to different effects. In view of the above theories, our research group used bioinformatics to screen for 12 single nucleotide polymorphisms (SNPs), including rs1045481, rs1045491, rs2740349, rs10719, rs642321, rs3744741, rs7813, rs3742330, rs3757, rs14035, rs720012, and rs4961280, which are derived from 6 main genes (i.e., GEMIN4, DICER1, DROSHA, DGCR8, AGO2, and RAN) acting in the microRNA-machinery pathway. We then analyzed the correlations between TB patients of Uygur in Xinjiang China and the above SNPs using a case-control study. The results showed that the genotypic distributions of rs720012 (from gene DGCR8) and rs4961280 (from gene AGO2) were not in accordance with the Hardy–Weinberg equilibrium ($P<.05$), so they were deleted. Subjects carrying the rs3742330 GG/GA genotype, rs1045481 GA genotype, rs1045491 CT genotype, and rs7813 AG genotype, respectively, had an increased risk of TB than individuals carrying rs3742330 AA genotype, rs1045481 GG/AA genotype, rs1045491 CC/TT genotype, and rs7813 AA/AG genotype between different groups. Expression quantitative trait loci analysis found that rs3744741 and rs2740349 from gene GEMIN4 had a regulatory effect, while rs3742330 from gene DICER1 had a reverse regulatory effect. Finally, according to the results of Linkage Disequilibrium between SNPs, the haplotype analysis showed that the haplotype of GCTAC from gene GEMIN4 had statistical differences when compared with active and inactive TB. The current experimental results provide a direction for our future research, and the research team will conduct more in-depth studies on the correlation between miRNA and TB.

Abbreviations: eQTL = expression quantitative trait loci, HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, PCR = polymerase chain reaction, SNP = single nucleotide polymorphism, TB = tuberculosis.

Keywords: microRNA, single nucleotide polymorphism, Uygur, tuberculosis, DICER1, GEMIN4

1. Introduction
Tuberculosis (TB), a major global health problem, causes ill-health in millions of people each year, and it is the second-leading cause of death by infectious diseases worldwide. The latest estimate in 2011 showed almost 9 million new TB cases and 1.4 million TB-related deaths in 1 year.\textsuperscript{[1]} Recently, TB has been controlled by the improvement of sanitation and the development of anti-TB agents. However, TB remains a serious health problem due to the emergence of multidrug-resistant strains and coinfection with TB and human immunodeficiency virus.\textsuperscript{[2,3]}

As a region of high TB prevalence, China’s public health problems remain serious. The TB mortality rate in Xinjiang is 2.9 times the national average. Xinjiang Uygur Autonomous Region (Xinjiang), in northwest China, has one of the highest rates of incidence of TB and mortality from TB. The Uygur and Kazak populations in the Xinjiang Uygur Autonomous Region of northwest China are seriously affected by TB. In the fourth Xinjiang TB epidemiological survey, the prevalence rate of active, sputum smear and culture positive TB in the Uygur, Kazak, and Mongolian populations of the Xinjiang Uygur Autonomous Region (northwest China) was found to be 12.4%, 16.9%, and 18.4%, respectively, higher than the Chinese Han population.\textsuperscript{[3]}

The Uygur in Xinjiang live in the northwest of China, and the prevalence and mortality of TB are significantly higher in the Uygur population than in the Han and other minorities in China.\textsuperscript{[4–6]} TB patients in Xinjiang are mainly located in southern Xinjiang, such as the Kashgar Prefecture, Hotan region, and Aksu region, and approximately 60% of the Uygur patients are farmers and herdsmen.\textsuperscript{[7]} The article suggests the following reasons for high prevalence: special geographical environment, arid climate, unique customs, and lifestyle; reliable evidence suggests that host genetic factors are involved in TB susceptibility. There is ethnic difference between the polymorphisms of candidate genes and TB susceptibility\textsuperscript{[8,9]}, and after natural selection, Uygur has developed some genetic features that are
different from those populations in other areas. A specific hereditary background may influence the host response to disease, and several reports have shown that host genetic factors play a significant role in susceptibility to TB.\cite{10,11,12}

According to the literature, the genetic factors evaluated in previous studies were mainly focused on coding proteins that are associated with the host immune system, including toll-like receptors 2 (TLR2),\cite{13} vitamin D receptor,\cite{14} and natural resistance associated macrophage protein 1 (N.RAMP).\cite{15} However, the influence of miRNA mechanical genes on susceptibility to TB is rarely appreciated.

MiRNA is a new single-stranded RNA approximately 22 nucleotides in length. It is produced by an independent non-coding region RNA or protein-coding genes intron transcription, and it has been found that 50% to 60% of the genes are regulated by miRNA.\cite{16} MiRNA plays an important role in the interaction between Mycobacterium tuberculosis and the host. Recent studies have shown that miRNAs are key regulators of gene expression.\cite{17} Although only a few hundred miRNAs have been discovered, each miRNA could potentially regulate hundreds of target genes. It has been suggested that one-third of human genes may be regulated by miRNAs.\cite{18} The expression levels of miRNAs have also been shown to be associated with cancer\cite{19} and other diseases.\cite{20} In addition, miRNAs have been used as potential biomarkers for both noncommunicable and communicable diseases.\cite{21,22} The altered gene expression profiles in natural killer cells and macrophages from TB-infected patients and healthy controls are also regulated by miRNAs.\cite{23}

In mammals, canonical miRNAs are generally transcribed as immature (stem-loop containing) precursor RNAs that are cleaved by 2 RNAse-III proteins: DROSHA in the nucleus and Dicer1 in the cytoplasm. Conditional genetic knockouts of essential moieties for miRNA biogenesis, such as the “Microprocessor complex” (composed by the type III ribonuclease DROSHA and the RNA-binding protein DiGeorge syndrome critical region gene 8 [DGCR8], also known as PASHA), or the type III ribonuclease Dicer1\cite{24,25,26} have been widely used to infer the global role of miRNAs in murine corticogenesis. The transcripts are processed by a multiprotein complex that includes DROSHA to form precursor miRNA (pre-miRNA) hairpins (about 60–100 nucleotides). After pre-miRNA has been exported to the cytoplasm by Ran GTPase (RAN) and exportin 5 (XPO5), it is further processed by Dicer1, a polymerase II enzyme. GEMIN4 protein is accepted as a key member of the GEMIN protein family that is involved in multiple pathological processes. It was reported that this protein was a shared part of the survival of motor neuron complex and a 15S ribonucleoprotein complex (miRNPs). The GEMIN4 protein was also referred to as an important molecule in the RNA-induced silencing complex that participated in the mature process of miRNAs, the target RNA recognition and repression.\cite{27}

From what has been discussed above, the current pathogenesis of TB is still unclear, and there are few studies on the diversity of large samples. In this article, a total of 465 cases of TB and 310 healthy controls were collected from the Uygur population of Xinjiang China. All subjects were tested for single nucleotide polymorphisms (SNPs) using a case-control study, which lays a foundation for further study on the mechanisms of miRNA-related TB susceptibility in Xinjiang Uygur.

2. Materials and methods

2.1. Study populations

The experiment section: Including the case group and the control group.

Case group: A total of 465 confirmed hospitalized patients were sampled from the Kashgar, Hotan, and Aksu autonomous prefecture of Xinjiang. None of the patients received anti-TB treatment or had other infectious diseases. The patients met one of the following conditions: Consistent evidence of clinical and imaging findings suggestive of TB and at least 2 different sputum smears from separate occasions were positive; positive culture of M tuberculosis in the sputum, bronchial lavage, or pleural fluid; and a biopsy found pathological evidence of TB.

Control group: A total of 310 healthy controls from the above areas. Age and sex were roughly matched with case group (Table 1).

The verification section: Including 25 confirmed hospitalized patients and 25 healthy patients, samples from TB patients were collected from the Hotan Tuberculosis Hospital, and the diagnostic criteria were the same as for the case group. Healthy patients came from the health check-up population in Hotan.

All the subjects were Uygur and had lived in the area for a long time; they have similar customs and living conditions. The Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University approved the present study. All subjects were provided written informed consent for sample collection and poststudy analysis.

2.2. SNP selection in the microRNA-machinery pathway

Comprehensive analysis of the literature reports on the pathogenesis of TB and bioinformatics screened out the main genes acting in the microRNA-machinery pathway (i.e., GEMIN4, DICER1, DROSHA, DGCR8, AGO2, and RAN). Scanning the databases of the International HapMap Project (http://www.hapmap.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and miRBase registry (http://microrna.sanger.ac.uk), we identified 12 potential functional polymorphisms in the microRNA-machinery pathway. All SNPs have a reported minor allele frequency (MAF) of more than 0.1 in Chinese patients (MAF > 10%).

2.3. Genotype and allelic analysis of SNPs

Peripheral blood samples (3mL/individual) from patients and healthy controls were stored in aseptic anticoagulant tubes at 70°C. Genomic DNA was extracted from blood samples using a

| Table 1 | Epidemiologic data of enrolled subjects. |
| --- | --- |
| Characteristics | Cases (465) |
| --- | --- |
| Age, years range | 33.21±13.8 | 37.43±17.2 | 38.73±13.15 |
| Gender | | | |
| Male | 82 | 177 | 170 |
| Female | 73 | 133 | 140 |
| SD = standard deviation. | | | |
Qiagen DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Briefly, blood samples were digested and further purified with protease K and phenol-chloroform, respectively. The genotyping of SNPs was analyzed by the Center for Genetic and Genomic Analysis, Genesky Biotechnologies Inc, Shanghai. The genotype method used was the improved multiplex ligase detection reaction (IMLDR) method. The conditions for the polymerase chain reaction (PCR) assay were: a final reaction volume of 10 µL which contained GC-ligase-Takara (1 ×), 3.0 mM Mg²⁺, 0.3 mM dNTP, 1 U HotStarTaq polymerase (Qiagen), 1 µL sample DNA, and 1 µL PCR primer. The procedure for the PCR cycle was as follows: 95°C for 2 minutes, 11 cycles at 92°C for 20 seconds, 65°C for 40 seconds, 72°C for 1.5 minutes, 24 cycles at 94°C for 20 seconds, 59°C for 30 seconds, 72°C for 1.5 minutes, 72°C for 2 minutes, and 4°C forever. The system for the DNA ligase reactions was as follows: 1 µL ligase buffer (10 ×), 0.25 µL ligase enzyme, 0.4 µL 5’ primer (1 µM), 0.4 µL 3’ primer (2 µM), 2 µL PCR product, and 6 µL ddH₂O. The procedure for the DNA ligase reaction was 94°C for 1 minute, 56°C for 4 minutes, and 4°C forever. The ABI 3730XL sequence detection system (Applied Biosystems) was used to analyze the SNPs on FCRL3. Genotyping data were analyzed by Gene Mapper 4.1 (Applied Biosystems, Thermo Fisher Scientific Company, China). We applied the double-control approach to ensure the accuracy of genotyping, and 5% of the samples were randomly selected so that the results were 100% accurate.

2.4. Statistical analysis

Hardy–Weinberg equilibrium (HWE) was determined using the goodness-of-fit chi-squared test. Differences in categorical variables such as genotype and allele frequencies in both the cases and controls were tested using a Pearson chi-squared test. Unconditional logistic regression analysis was performed to investigate the relationship between the risk of TB and the SNPs after controlling of sex and age. All the above analysis processes were mainly based on PLINK analysis. Expression quantitative trait loci analysis (eQTL) was used to complete the verification. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Basic demographic characteristics of the subjects

Test subjects were divided into the case group (465 cases) and the control group (310 cases). The case group was further divided into active TB and inactive TB. Verification subjects included 25 confirmed hospitalized patients and 25 healthy people. The subjects were not statistically different in terms of sex and age.

3.2. Hardy–Weinberg balance control

Twelve SNP genotypes were detected in both the cases and controls, the genotyping rate and the minimum allele frequency of the 12 SNPs were recorded in Table 2. The genotype distributions of rs720012 and rs4961280 were not consistent with HWE (P < 0.05, and there was no H-W balance in the group), so we deleted the genotypes without subsequent analysis. The results of the Linkage Disequilibrium between SNPs were in Fig. 1 (A–C).

3.3. Comparison of cases and controls

Except the genotype distribution of rs720012 and rs4961280, other SNPs were tested in both cases and controls (details in Table 3). A Pearson chi-squared test was performed to investigate the categorical variables of allele frequencies, and unconditional logistic regression analysis was performed to investigate the relationship between the risk of TB and SNPs of different genotypes. The analysis models include codominant, dominant, recessive, and additive.

Logistic regression analysis demonstrated that subjects carrying the rs3742330 AG/GG genotype had a significantly increased risk for TB than individuals carrying the AA genotype in the model of dominant analysis (OR = 1.37, 95% CI = 1.02–1.83, P = 0.03). Moreover, log-additive analysis was also statistically significant (OR = 1.33, 95% CI = 1.04–1.72, P = 0.02). A chi-squared test demonstrated that subjects carrying the G allele of the rs3742330 have an increased risk for TB than A allele (OR = 1.33, 95% CI = 1.04–1.70, P = 0.02).

3.4. Comparison of active TB and controls

After comparing active TB cases (155 cases) and healthy controls (310 cases), we found the following: Subjects with the rs1045481 GA genotype had a significantly increased risk for TB than individuals carrying the GG and AA genotype in the model of codominant analysis (OR = 1.66, 95% CI = 1.07–2.58, P = 0.03), and it also has statistically significant in the model of dominant and additive. Carrying the A allele of rs1045481 than G can also

### Table 2

| SNP       | CHR | Position (hg19) | Ref allele | Alt allele | Gene name | Region | HWE       | Total MAF | Case MAF | Control MAF |
|-----------|-----|-----------------|------------|------------|-----------|--------|-----------|-----------|----------|-------------|
| n1045481  | 17  | 648157          | G          | A          | GEMIN4    | Exonic | 1         | 0.13      | 0.14     | 0.11        |
| n1045491  | 17  | 647988          | C          | T          | GEMIN4    | UTR3   | 0.34      | 0.13      | 0.14     | 0.12        |
| n2740349  | 17  | 646988          | T          | C          | GEMIN4    | UTR3   | 0.72      | 0.18      | 0.17     | 0.20        |
| n10719    | 5   | 31401447        | G          | A          | DROSHA   | UTR3   | 0.83      | 0.49      | 0.49     | 0.49        |
| n642321   | 5   | 31401003        | C          | T          | DROSHA   | UTR3   | 1         | 0.35      | 0.35     | 0.36        |
| n3744741  | 17  | 649232          | C          | T          | GEMIN4    | Exonic | 0.92      | 0.23      | 0.24     | 0.23        |
| n7813     | 17  | 648186          | A          | G          | GEMIN4    | Exonic | 0.75      | 0.35      | 0.35     | 0.35        |
| n720012   | 22  | 20098582        | G          | A          | DCCR8    | UTR3   | 0.01      | 0.26      | 0.26     | 0.27        |
| n3742330  | 14  | 95553362        | A          | G          | DICER1   | UTR3   | 0.49      | 0.23      | 0.25     | 0.20        |
| n3757     | 22  | 20099331        | G          | A          | DCCR8    | UTR3   | 0.44      | 0.25      | 0.25     | 0.24        |
| n142885   | 12  | 131361241       | C          | T          | RAN      | UTR3   | 0.73      | 0.19      | 0.20     | 0.19        |
| n4061280  | 8   | 141647414       | C          | A          | AG02     | Intergenic | 0.03    | 0.17      | 0.16     | 0.17        |

All allele = another allele of SNP, CHR = the chromosome number, HWE = Hardy–Weinberg equilibrium, MAF = minimum allele frequency, Position (hg19) = the location of SNP on the chromosome in version hg19, Ref allele = the allele of SNP on reference sequence, Region = the region on the gene, SNP = single nucleotide polymorphism, Total MAF = MAF in all samples.

* P < 0.05 is in bold.
increase risk for TB (OR = 1.49, 95% CI = 1.0 – 2.2, \( P = .04 \)) (details in Table 4). Subjects carrying the rs1045491 CT genotype had a significantly increased risk for TB than individuals carrying the CC and TT genotype in the model of codominant analysis (OR = 1.67, 95% CI = 1.07 – 2.61, \( P = .02 \)), carrying the rs1045491 CT/TT genotype had a significantly increased risk for TB than individuals carrying the CC genotype in the model of dominant analysis (OR = 1.62, 95% CI = 1.05 – 2.50, \( P = .03 \)).
Table 3
Comparisons of gene polymorphisms between cases (465) and controls (310).

| Gene name/polymorphism | Model       | Cases, N (%) | Controls, N (%) | P-corr   | OR       | 95% CI       | FDR_BH adjusted |
|------------------------|-------------|--------------|-----------------|----------|----------|--------------|-----------------|
| GEMIN4                 | Codominant  | G/G 347 (74.62) 243 (78.39) | 1 |  | | | |
|                        |             | G/A 110 (23.66) 62 (20.00) | .26 1.22 0.86–1.74 | 0.84 | | | |
|                        |             | A/A 8 (1.72) 5 (1.61) | .59 1.40 0.42–4.70 | 0.84 | | | |
| Dominant               | G/G 347 (74.62) 243 (78.39) | 1 |  | | | | |
|                        | G/A-A/A 118 (25.38) 67 (21.61) | .23 1.23 0.88–1.74 | 0.71 | | | | |
|                        | A/A 8 (1.72) 4 (1.29) | .64 1.34 0.40–4.49 | 0.91 | | | | |
| Additive               | G 804 (86.45) 549 (88.55) | 1 |  | | | | |
| Allele                 | A 126 (13.55) 71 (11.45) | .22 1.21 0.89–1.66 | 0.75 | | | | |
| GEMIN4                 | Codominant  | C/C 348 (74.84) 242 (78.06) | 1 |  | | | |
|                        |             | C/T 151 (31.87) 99 (31.94) | .29 0.81 0.59–1.12 | 0.71 | | | |
|                        |             | C/C 14 (3.01) 14 (4.51) | .21 0.62 0.29–3.23 | 0.93 | | | |
| Dominant               | C/C 348 (74.84) 242 (78.06) | 1 |  | | | | |
|                        | C/T-T/T 117 (25.16) 68 (21.94) | .27 1.21 0.86–1.71 | 0.71 | | | | |
|                        | T/T 10 (2.15) 7 (2.26) | .84 1.11 0.40–3.09 | 1 | | | | |
| Additive               | C 803 (86.34) 545 (87.90) | 1 |  | | | | |
| Allele                 | T 127 (13.66) 75 (12.10) | .29 1.17 0.88–1.59 | 0.75 | | | | |
| DROSHA                | Codominant  | G/G 119 (25.59) 80 (25.81) | 1 |  | | | |
| rs10719                |             | G/A 235 (50.54) 156 (50.32) | .94 1.01 0.71–1.43 | 0.99 | | | |
|                        |             | A/A 111 (23.87) 74 (23.87) | .97 1.00 0.67–1.52 | 0.99 | | | |
| Dominant               | G/G 119 (25.59) 80 (25.81) | 1 |  | | | | |
|                        | G/A-A/A 131 (28.17) 99 (31.94) | .20 0.81 0.59–1.12 | 0.71 | | | | |
|                        | A/A 14 (3.01) 14 (4.51) | .21 0.62 0.29–3.23 | 0.93 | | | | |
| Recessive              | G/G-A/A 131 (28.17) 99 (31.94) | 1 | | 1 | | | | |
|                        | T/T 10 (2.15) 7 (2.26) | .84 1.11 0.40–3.09 | 1 | | | | |
| Additive               | G 473 (50.86) 316 (50.97) | 1 |  | | | | |
| Allele                 | T 159 (17.10) 127 (20.48) | .09 0.80 0.62–1.04 | 0.82 | | | | |
| DROSHA                | Codominant  | G/G 119 (25.59) 80 (25.81) | 1 |  | | | |
| rs642321               |             | G/A 235 (50.54) 156 (50.32) | .94 1.01 0.71–1.43 | 0.99 | | | |
|                        |             | A/A 111 (23.87) 74 (23.87) | .97 1.00 0.67–1.52 | 0.99 | | | |
| Dominant               | G/G 119 (25.59) 80 (25.81) | 1 |  | | | | |
|                        | G/A-A/A 131 (28.17) 99 (31.94) | .20 0.81 0.59–1.12 | 0.71 | | | | |
|                        | A/A 14 (3.01) 14 (4.51) | .21 0.62 0.29–3.23 | 0.93 | | | | |
| Recessive              | G/A-G/A 131 (28.17) 99 (31.94) | 1 | | 1 | | | | |
|                        | T/T 10 (2.15) 7 (2.26) | .84 1.11 0.40–3.09 | 1 | | | | |
| Additive               | C 609 (65.48) 316 (60.97) | 1 |  | | | | |
| Allele                 | T 457 (49.14) 304 (49.03) | .97 1.00 0.82–1.23 | 0.97 | | | | |
| GEMIN4                 | Codominant  | C/C 270 (58.07) 183 (59.03) | 1 |  | | | |
| rs3744741              |             | C/T 169 (36.34) 108 (34.64) | .71 1.06 0.78–1.44 | 0.99 | | | |
|                        |             | T/T 26 (5.59) 19 (6.13) | .91 1.04 0.55–1.97 | 0.99 | | | |
| Dominant               | C/C 270 (58.07) 183 (59.03) | 1 |  | | | | |
|                        | C/T-T/T 195 (41.94) 125 (40.32) | .71 1.06 0.79–1.42 | 0.83 | | | | |
|                        | T/T 26 (5.59) 19 (6.13) | .91 1.04 0.55–1.97 | 0.99 | | | | |
| Additive               | C 709 (76.24) 476 (76.77) | 1 |  | | | | |
| Allele                 | T 321 (34.52) 223 (35.97) | .56 0.94 0.76–1.16 | 0.93 | | | | |
| GEMIN4                 | Codominant  | A/A 194 (41.72) 129 (41.61) | 1 |  | | | |
| rs7813                 |             | A/G 216 (46.45) 142 (45.81) | .94 1.01 0.74–1.38 | 0.93 | | | |
|                        |             | G/G 55 (11.83) 39 (12.58) | .79 0.94 0.50–1.50 | 0.93 | | | |
| Dominant               | A/A 194 (41.72) 129 (41.61) | 1 |  | | | | |
|                        | A/G-G/G 216 (46.45) 142 (45.81) | .94 1.01 0.74–1.38 | 0.93 | | | | |
|                        | G/G-G/G 55 (11.83) 39 (12.58) | .79 0.94 0.50–1.50 | 0.93 | | | | |
| Recessive              | A/A-A/G 216 (46.45) 142 (45.81) | 1 | | 1 | | | | |
|                        | A/G-G/G 55 (11.83) 39 (12.58) | .79 0.94 0.50–1.50 | 0.93 | | | | |
|                        | G/G-G/G 55 (11.83) 39 (12.58) | .79 0.94 0.50–1.50 | 0.93 | | | | |

(continued)
| Gene name/polymorphism | Model | Cases, N (%) | Controls, N (%) | P-corr | OR 95% CI | FDR_BH adjusted |
|------------------------|-------|--------------|----------------|--------|-----------|----------------|
| **DICER1 rs3742330**   |       |              |                |        |           |                |
| Additive               | G/G   | 55 (11.83)   | 39 (12.58)     | .75    | 0.93      | 0.60–1.45      | 1               |
| Allele                 | A     | 604 (64.95)  | 400 (64.52)    | .86    | 0.96      | 0.79–1.22      | 0.96            |
|                       | G     | 326 (35.05)  | 220 (35.48)    | .86    | 0.98      | 0.79–1.21      | 0.99            |
| Codominant            | A/A   | 256 (55.05)  | 194 (62.58)    | 1      |           |                |                |
|                       | A/G   | 181 (38.92)  | 105 (33.87)    | .09    | 1.31      | 0.96–1.77      | 0.71            |
|                       | G/G   | 28 (6.02)    | 11 (3.55)      | .07    | 1.93      | 0.94–3.97      | 0.71            |
| Dominant              | A/A   | 256 (55.05)  | 194 (62.58)    | 1      |           |                |                |
|                       | A/G   | 209 (44.95)  | 116 (37.42)    | .03    | 1.37      | 1.02–1.83      | 0.70            |
|                       | G/G   | 437 (93.98)  | 299 (96.45)    | .02    | 1.33      | 1.04–1.72      | 0.43            |
| Recessive             | A/A   | 55 (11.83)   | 39 (12.58)     | .05    | 1.46      | 0.99–2.17      | 0.62            |
|                       | A/G   | 604 (64.95)  | 400 (64.52)    |      |           |                |                |
|                       | G/G   | 326 (35.05)  | 220 (35.48)    |      |           |                |                |
| Allele                | G     | 604 (64.95)  | 400 (64.52)    |      |           |                |                |
|                       | A     | 326 (35.05)  | 220 (35.48)    |      |           |                |                |
|                       |       |              |                |        |           |                |                |
| **DGCR8 rs3757**       |       |              |                |        |           |                |
| Additive              | G/G   | 604 (64.95)  | 400 (64.52)    |      |           |                |                |
| Allele                | G     | 326 (35.05)  | 220 (35.48)    |      |           |                |                |
|                       | A     | 604 (64.95)  | 400 (64.52)    |      |           |                |                |
|                       |       |              |                |        |           |                |                |
| **RAN rs14035**        |       |              |                |        |           |                |
| Additive              | G/G   | 604 (64.95)  | 400 (64.52)    |      |           |                |                |
| Allele                | G     | 326 (35.05)  | 220 (35.48)    |      |           |                |                |
|                       | A     | 604 (64.95)  | 400 (64.52)    |      |           |                |                |
|                       |       |              |                |        |           |                |                |

95% CI = 95% confidence interval, OR = odds ratio.
*<.05 is in bold.

**Table 4**
Comparisons of polymorphisms between the active tuberculosis (155) and controls (310).

| Gene name/polymorphism | Model | Active, N (%) | Controls, N (%) | P-corr | OR 95% CI | FDR_BH adjusted |
|------------------------|-------|--------------|----------------|--------|-----------|----------------|
| **GEMIN4 rs1045481**   |       |              |                |        |           |                |
| Codominant            | G/G   | 107 (69.03)  | 243 (78.39)    | 1      |           |                |                |
|                       | G/A   | 46 (29.68)   | 63 (20.32)     | .03    | 1.66      | 1.07–2.58      | 0.95            |
|                       | A/A   | 2 (1.29)     | 4 (1.29)       | .88    | 1.14      | 0.20–6.29      | 0.95            |
| Dominant              | G/G   | 107 (69.03)  | 243 (78.39)    | 1      |           |                |                |
|                       | G/A   | 46 (29.68)   | 63 (20.32)     | .03    | 1.63      | 1.05–2.51      | 0.30            |
|                       | A/A   | 2 (1.29)     | 4 (1.29)       | 1      | 1         | 0.18–5.52      | 1               |
| Recessive             | G/G   | 153 (96.71)  | 306 (98.71)    |      |           |                |                |
|                       | G/A   | 2 (1.29)     | 4 (1.29)       |      | 1         | 0.18–5.52      | 1               |
|                       |       |              |                |        |           |                |                |
| **GEMIN4 rs1045491**   |       |              |                |        |           |                |
| Codominant            | C/C   | 107 (69.03)  | 243 (78.39)    | 1      |           |                |                |
|                       | C/T   | 45 (29.03)   | 62 (20.0)      | .02    | 1.67      | 1.07–2.61      | 0.95            |
|                       | T/T   | 3 (1.94)     | 6 (1.94)       | .86    | 1.13      | 0.28–4.61      | 0.95            |
| Dominant              | C/C   | 107 (69.03)  | 243 (78.39)    | 1      |           |                |                |
|                       | C/T   | 45 (29.03)   | 62 (20.0)      | .03    | 1.62      | 1.05–2.50      | 0.30            |
|                       | T/T   | 3 (1.94)     | 6 (1.94)       | .99    | 0.99      | 0.25–4.04      | 1               |
| Recessive             | C/C   | 152 (98.06)  | 303 (97.4)     |      |           |                |                |
|                       | C/T   | 48 (30.30)   | 68 (21.94)     |      | 1         | 0.24–12.04     | 0.09            |
|                       | T/T   | 1 (0.62)     | 2 (0.65)       | .05    | 1.46      | 0.89–12.04     | 0.09            |
|                       |       |              |                |        |           |                |                |

95% CI = 95% confidence interval, OR = odds ratio.
*<.05 is in bold.
3.5. Comparison of active TB and inactive TB

We compared active TB (155 cases) with inactive TB (310 cases) and found the following: Subjects carrying the rs1045481 GA genotype had a significantly increased risk for TB than individuals carrying the GG and AA genotype in the model of codominant analysis (OR = 1.61, 95% CI = 1.04–2.51, *P* = .03) (details in Table 5). Subjects carrying the rs1045491 CT genotype had a significantly increased risk for TB than individuals carrying the CC and TT genotype in the model of codominant analysis (OR = 1.64, 95% CI = 1.05–2.55, *P* = .03). Subjects carrying the rs7813 AG genotype had a significantly increased risk for TB than individuals carrying the AA and GG genotype in the model of codominant analysis (OR = 1.66, 95% CI = 1.09–2.52, *P* = .02).

### Table 5

| Gene name/polymorphism | Model  | Active, N (%) | Inactive, N (%) | P-corr | OR      | 95% CI     | FDR_BH adjusted |
|------------------------|--------|---------------|----------------|--------|---------|------------|-----------------|
| GEMIN4                 | Codominant |               |                |        |         |            |                 |
| rs1045481              | G/G    | 107 (69.03)   | 240 (77.42)    | 1      |         |            |                 |
|                        | G/A    | 46 (29.68)    | 64 (20.65)     | .03    | 1.61    | 1.04–2.51  | .09             |
|                        | A/A    | 2 (1.29)      | 6 (1.93)       | .72    | 0.75    | 0.15–3.77  | .09             |
| GEMIN4                 | Dominant |               |                |        |         |            |                 |
| rs1045481              | G/G    | 107 (69.03)   | 240 (77.42)    | 1      |         |            |                 |
|                        | G/A    | 48 (30.97)    | 70 (22.58)     | .05    | 1.54    | 0.99–2.37  | .34             |
| GEMIN4                 | Recessive |              |                |        |         |            |                 |
| rs1045481              | G/G-A/A | 153 (98.71)   | 304 (98.06)    | 1      |         |            |                 |
| GEMIN4                 | Additive |              |                |        |         |            |                 |
| rs1045481              | G/A    | 2 (1.29)      | 6 (1.94)       | .62    | 0.66    | 0.13–3.32  | .89             |

### Table 6

| Pheno name SNP | Gene name | Model  | Beta | SE   | 95% CI   | P-corr | FDR_BH adjusted |
|----------------|-----------|--------|------|------|----------|--------|-----------------|
| PIWIL1 _ELISA | rs3744741 | GEMIN4 | Dominant | 21.02 | 9.52 | 39.69–2.36 | .03 | 0.644 |
| PIWIL1 _ELISA | rs3744741 | GEMIN4 | Additive | 20.06 | 8.25 | 35.26–3.91 | .01 | 0.474 |
| GEMIN4_OAZ1   | rs3740349 | GEMIN4 | HET | 0.0019 | 0.0086 | 0.0004 to 0.002 | .03 | 0.576 |
| GEMIN4_OAZ1   | rs3742330 | DICER1 | HOM | −0.0020 | 0.0085 | −0.003 to 0.004 | .02 | 0.474 |
| GEMIN4_OAZ1   | rs3742330 | DICER1 | Recessive | −0.0020 | 0.0088 | −0.003 to 0.004 | .02 | 0.58 |

95% CI = 95% confidence interval, Beta = regression coefficient, SE = standard error, SNP = single nucleotide polymorphism.

<.05 is in bold.

3.6. Verification test

According to the above results, the gene GEMIN4 (rs1045481, rs1045491, and rs7813) and DICER1 (rs3742330) were statistically significant between different groups (details in Table 6). To further verify the correlation between the above SNPs and gene expression, another 50 samples (including 25 cases and 25 controls) were collected. All 10 SNPs were analyzed based on eQTL. We found that rs3744741 and rs2740349 in GEMIN4 had a regulatory effect, while rs3742330 in DICER1 had a reverse regulatory effect, the detailed results were shown in Table 6. Moreover, Fig. 2(A–E) were the boxplots showed the expression levels in the top SNPs.
Figure 2. The boxplots of the expression levels in the top SNPs. (A) Expression levels of rs2740349 from gene GEMIN4 with the model of HET. (B) Expression levels of rs3742330 from gene GEMIN4 with the model of Recessive, and (C) was that with the model of HOM. (D) Expression levels of rs3744741 from gene GEMIN4 with the model of Dominant, and (E) was that with the model of Additive. SNP = single nucleotide polymorphism.
Gene regulation mechanisms and silencing of host miRNAs may unlikely to have important functions,[30] TB is susceptible to host in relevant gene regions that combine with miRNA are rare and the proliferation of T cells.[29] In addition, although the SNPs in anti-TB regulation by changing the production of cytokines [85]

Hap SNPs Group Haplotype Case_F Control_F OR 95% CI P-corr

| Hap | SNPs | Group | Haplotype | Case_F | Control_F | OR  | 95% CI     | P-corr |
|-----|------|-------|-----------|--------|-----------|-----|-----------|--------|
| DRGHA | rs642321, rs10719 | Cases vs controls | CG | 473 (0.51) | 316 (0.51) | 0.89 | 0.81–1.22 | .96 |
| TA | 321 (0.35) | 223 (0.38) | 0.94 | 0.76–1.16 | .55 |
| CA | 136 (0.19) | 81 (0.13) | 1.14 | 0.85–1.53 | .38 |
| Active vs controls | CG | 162 (0.52) | 316 (0.51) | 1.05 | 0.80–1.38 | .71 |
| TA | 102 (0.33) | 223 (0.36) | 0.87 | 0.651,165 | .35 |
| CA | 46 (0.15) | 81 (0.13) | 1.16 | 0.78–1.71 | .45 |
| Active vs inactive | CG | 162 (0.52) | 311 (0.50) | 1.09 | 0.83–1.43 | .54 |
| TA | 102 (0.33) | 219 (0.35) | 0.90 | 0.67–1.19 | .46 |
| CA | 46 (0.15) | 90 (0.15) | 1.03 | 0.69–1.51 | .89 |

| GEMIN4 | rs1045481, rs3744741, rs2740349, rs7813, rs1045491 | Cases vs controls | GCTGC | 42 (0.05) | 21 (0.03) | 1.35 | 0.79–2.30 | .27 |

| Haplotype | Case_F | Control_F | OR  | 95% CI     | P-corr |
|-----------|--------|-----------|-----|-----------|--------|
| GCTAC | 380 (0.41) | 258 (0.42) | 0.97 | 0.79–1.19 | .76 |
| GTAC | 221 (0.24) | 142 (0.23) | 1.05 | 0.82–1.33 | .69 |
| GCSC | 158 (0.17) | 125 (0.20) | 0.81 | 0.62–1.05 | .11 |
| ACTGT | 124 (0.13) | 71 (0.12) | 1.19 | 0.87–1.62 | .27 |
| Active vs controls | GCTGC | 17 (0.06) | 21 (0.03) | 1.66 | 0.86–3.18 | .13 |
| GCTAC | 112 (0.36) | 258 (0.42) | 0.79 | 0.60–1.05 | .10 |
| GTAC | 76 (0.25) | 142 (0.23) | 1.09 | 0.79–1.50 | .58 |
| GGCSC | 54 (0.17) | 125 (0.20) | 0.84 | 0.59–1.19 | .31 |
| ACTGT | 50 (0.16) | 71 (0.12) | 1.19 | 0.71–2.04 | .20 |
| Active vs inactive | GCTGC | 47 (0.06) | 29 (0.04) | 1.38 | 0.76–2.05 | .03 |
| GCTAC | 112 (0.36) | 268 (0.43) | 0.74 | 0.56–0.98 | .03 |
| GTAC | 76 (0.25) | 145 (0.23) | 1.06 | 0.77–1.46 | .70 |
| GGCSC | 54 (0.17) | 104 (0.17) | 1.05 | 0.73–1.50 | .80 |
| ACTGT | 50 (0.16) | 74 (0.12) | 1.42 | 0.96–2.09 | .07 |

95% CI = 95% confidence interval, OR = odds ratio, SNP = single nucleotide polymorphism.

The number and frequency of haplotypes in the case group.

The number and frequency of haplotypes in the control group.

<.05 is in bold.

3.7. Haplotype analysis

According to the results of Linkage Disequilibrium between SNPs in Fig. 1(A–C), the blocks with strong correlation were found to analyzed the haplotype, and then conducted the correlation analysis by logistic regression (details in Table 7). The results showed that the haplotype of GCTAC in GEMIN4 gene had statistical differences when compared with active and inactive TB (OR = 0.74, 95% CI = 0.56–0.98, P = .03).

4. Discussion

At present, there are relatively few reports regarding the susceptibility of Uygur TB patients in China. This article collected 465 cases of TB and 310 cases of healthy patients from Xinjiang China. Such a large study is rare. According to the current experimental results, we found that 4 SNPs (rs3742330, rs1045481, rs1045491, and rs7813) in 2 genes (DICER1 and GEMIN4) are associated with susceptibility to TB in the Uygur.

Both genetic factors and environmental factors can influence TB, and considering the unique genetic characteristics of the Uygur, we speculate that the incidence of TB in the Uygur is different from that in the Han nationality. Relevant evidence confirms that mRNA is associated with TB. MiR-144 is involved in anti-TB regulation by changing the production of cytokines and the proliferation of T cells.[29] In addition, although the SNPs in relevant gene regions that combine with miRNA are rare and unlikely to have important functions,[30] TB is susceptible to host gene regulation mechanisms and silencing of host miRNAs may be a mechanism for human macrophages to protect against TB.[11] Recent research shows that gene mutation in the seed sequence of miRNA genes may affect the treatment of miRNA and lead to a decrease in miRNA expression.[12] SNPs located in the 5/-UTR region may change the affinity of transcription factors and promoters during transcription, while SNPs in exogenous regions or introns can affect the conformation and function of proteins or precursor mRNA splicing.[13] Therefore, SNPs in mRNA target genes play an important role in the development of TB by changing the structure and function of miRNAs.

The SNP rs7813 is located at position Cys1033Arg in the GEMIN4 gene. Wan found that rs7813 was significantly associated with cell growth and DNA repair in a hepatocellular carcinoma cell line.[13] In addition, other studies have revealed a correlation between this gene mutation and the development of bladder cancer or a reduced risk for renal cell carcinoma.[14] Our study found that when comparing active TB with inactive TB, subjects carrying the rs7813 AG genotype had a significantly increased risk for TB than individuals carrying the AA and GG genotype in the model of codominant analysis. Subjects carrying the rs1045481 GA and rs1045491 CT genotypes can also increase the risk for TB compared with the GG/AA and TT/CC genotypes in the model of codominant analysis, Rs1045481 and rs1045491 are located in an exon and the 3’-UTR region of GEMIN4, respectively. When comparing active TB with healthy controls, rs1045481 and rs1045491 also showed statistically significant differences. Subjects carrying the rs1045481 GA
transcriptional stability and contains multiple sites for the miR-632-mediated regulation, which is possibly involved in the depth studies on the correlation between miRNA and TB. However, the current experimental results provide a direction for our future research, and the research team will conduct more in-depth studies on this gene in the future.

As for rs3742330, computational modeling suggested that this polymorphism was located in the hsa-miR-632 potential target sequence in the DICER 3'-UTR. DICER is the core component of the DICER-containing complex, which plays an important role in the cytoplasmic processing of pre-miRNAs to mature miRNAs. More importantly, this 3'-UTR is important for mRNA transcriptional stability and contains multiple sites for the regulation of targeted miRNAs, binding of transcription factors, DNA methylation and histone modification. Rs3742330 has been identified as the target site of has-miR-3622a-5p and has-miR-5582-5p. From the results of our experiments, carrying the rs3742330 AG-GG genotype had a significantly increased risk for TB than individuals carrying the AA genotype in the model of dominant analysis, and carrying the G allele of rs3742330 can also increase risk for TB. The verification results also confirm that rs3742330 has a reverse regulatory effect on DICER1. Therefore, we speculate that rs3742330 may affect the potential function of DICER1 expression by destroying the stability of mRNA transcription. This would result in a similar reaction in downstream miRNAs and ultimately contribute to the development of a variety of diseases. Based on bioinformatics analysis, we found that rs3742330 is located in the prediction binding site (seed region) of human miR-632. The Uygur and Tibetans carrying the G allele of rs3742330 showed differential expression between the cases and controls. As a result, we assume that miR-632 combined with DICER1 mRNA transcription contains an A allele, which has a negative effect on DICER1 gene expression. On the contrary, the mRNA transcription containing a G allele will be disturbed, allowing increased DICER1 expression.

Taken together, our data suggest that common genetic changes in DICER1 may influence TB risk in the Uygur, likely through miR-632-mediated regulation, which is possibly involved in the pathogenesis of TB. These results have also been confirmed among Tibetans in China. Therefore, it is necessary to continue to study the relationship between this gene and TB. However, there are still many problems to be solved. Our research lacks a functional experiment for detailed molecular mechanisms. Our study is limited to polymorphisms, and the expression of related genes in the cases and controls remains to be further studied. However, the current experimental results provide a direction for our future research, and the research team will conduct more in-depth studies on the correlation between miRNA and TB.

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