Association of *ficolin*-1 and *ficolin*-3 gene variation and pulmonary tuberculosis susceptibility in a Chinese population

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Abstract

**Background:** The aim of our study was to estimate the association of *ficolin*-1 (*FCN1*) gene (rs10120023, rs1071583) and *ficolin*-3 (*FCN3*) gene (rs3813800, rs10794501) polymorphisms and pulmonary tuberculosis (PTB) susceptibility, as well as their several clinical features, in a Chinese population.

**Methods:** This study included a cohort of 489 PTB patients and 489 healthy controls, and the four SNPs were genotyped by improved multiple ligase detection reaction (iMLDR).

**Results:** We found that there were no significant differences regarding the allele and genotype frequencies of *FCN1* rs10120023, rs1071583 and *FCN3* rs3813800, rs10794501 between PTB patients and healthy controls (all \( p > 0.05 \)). The association of three main haplotypes (CC, CT, and TC) in *FCN1* and three main haplotypes (CT, GA, and GT) in *FCN3* with PTB susceptibility was also analyzed, and no significant association was detected (all \( p > 0.05 \)). In *FCN1*, the rs1071583 TT genotype was significantly associated with the occurrence of drug resistance in PTB patients (\( p = 0.040 \)). In addition, the GG genotype and G allele frequencies of rs3813800 in *FCN3* gene were significantly higher in PTB patients with pulmonary infection (\( p = 0.027, p = 0.020 \), respectively).

**Conclusions:** *FCN1* and *FCN3* genetic variation were not contributed to the pathogenesis of PTB in Chinese. While rs1071583 and rs3813800 variant might associate with several clinical characteristics of PTB.

**Key Words**

*FCN1*, *FCN3*, genetic polymorphisms, pulmonary tuberculosis

1 INTRODUCTION

The World Health Organisation's (WHO) Global tuberculosis report 2019 shows that approximately 10 million people develop tuberculosis (TB) patients each year. Among these patients, pulmonary TB (PTB) is the most common disease, which is a serious, chronic infectious disease mainly caused by the Mycobacterium tuberculosis (MTB) bacillus. At present, TB remains a major public health problem in the world and is considered to be the main cause of death caused by a single cause and pathogen, because of multi-drug resistant,
merger HIV infection, etc. According to previous studies, nearly a third of the world’s population was infected with Mtb, however only 10–15% of these population with the risk of progressing active disease in their lives. A series of factors had been proved to affect the development process of PTB disease, including nutrition, diabetes, immunosuppression, social economy, and environmental pollution. In addition, some studies also revealed the significance of genetic predisposition in the pathogenesis of PTB. For example, many single nucleotide polymorphisms (SNPs) in genes as IL-17A, toll-like receptors, nucleotide oligomerization domain 2, CD14, vitamin D nuclear receptor, and monocyte chemoattractant protein-1 had been reported to increase or decrease susceptibility to PTB. Therefore, host gene variation was closely related to the risk of PTB.

The complement system played a key role in the host defense against infectious pathogens and could promote opsonization of immune complexes and pathogens, leukocyte recruitment, and cytolyis. As an important pathway of complement system, the lectin pathway was activated by multiple pattern recognition molecules (PRM)—ficolins, mannos-binding lectin (MBL), and collectin-11 (CL-K1) in association with the MBL/ficolin-associated serine proteases (MASPs)—which have the important function to recognize the glycoproteins on the surface of the pathogen. Ficolin is innate immunity pattern recognition molecules and has three types in humans, including ficolin-1 (M-ficolin), ficolin-2 (L-ficolin), and ficolin-3 (H-ficolin). Ficolin-1 is encoded by FCN1 gene, which is located at chromosome 9q34, and mainly secreted by monocytes and neutrophils. Ficolin-2 is encoded by FCN2 gene, which is located at chromosome 9q34, and mainly produced by hepatocytes and secreted into the blood stream. Ficolin-3 is encoded by FCN3 gene on chromosome 1p36 and is secreted in plasma. Recent years, many scholars had analyzed the correlation between FCN gene polymorphism and multiply autoimmune, inflammatory, and infectious diseases.

A previous study examined the role of FCN2 gene variant in PTB; however, the FCN1 and FCN3 gene SNPs associated with PTB susceptibility had not yet been reported in Chinese population. This study aimed to explore the potential association between FCN1 gene (rs10120023 and rs1071583) and FCN3 gene (rs3813800 and rs10794501) and susceptibility to PTB in the Chinese population.

2 | MATERIALS AND METHODS

2.1 | Study participants

PTB patients were sequentially recruited from the Department of Tuberculosis at Anhui Chest Hospital. All the patients were diagnosed by specialist according to the following criteria: suspicious clinical symptoms, chest radiography, sputum and/or bronchoalveolar lavage fluid Mtb culture, microscopy for acid-fast bacilli (AFB), and effect of anti-TB treatment. The PTB patients with HIV positive, hepatitis, malignant tumor, and immune-compromised conditions were excluded from the study. Demographic and several clinical data including drug resistance, drug-induced liver damage (DILI), and pulmonary infection of PTB patients were recorded. Healthy controls without a history of TB, malignant tumor, and HIV were enrolled from health examine center in the same area, and all controls needed to be asymptomatic with negative sputum smear and culture, and normal chest radiograph.

The Ethics Committee of Anhui Chest Hospital approved our research proposal on the basis of complying with the Helsinki Declaration (K2020-005). After obtaining the informed consent, we began to collect peripheral blood samples and personal information of all participants.

2.2 | DNA extraction and genotyping

The early morning fasting blood sample of all study subjects was drawn by EDTA tubes. Then, genomic DNA was extracted from each 5 ml peripheral blood sample according to the standard procedures of the Flexi Gene-DNA Kit (Qiagen, Valencia, CA), and stored at −80℃ until genotyping.

We systematically searched the existing studies regarding the association between FCN1 and FCN3 genes polymorphisms and human disease. Then, we tried to look for the SNPs that were associated with human disease but had not been studied in PTB. In addition, we obtained genotype data of Han Chinese in Beijing from Ensembl genome browser 85 and CHBS_1000 g, and selected the tag SNPs using the pairwise option of the Haploview 4.0 software (Cambridge, MA, USA). Finally, we selected two SNPs (rs10120023 and rs1071583) of FCN1 and two tag SNPs (rs3813800 and rs10794501) of FCN3 for genotyping in PTB patients and healthy controls. In addition, these SNPs all met the following conditions: minor allele frequency (MAF) ≥ 0.05 in CHB, and r² threshold > 0.8.

In this study, improved multiple ligase detection reaction (iMLDR) was adopted for genotyping with the technical support of the Center for Genetic & Genomic Analysis, Genesky Biotechnologies (Inc., Shanghai). A multiplex PCR-ligase detection reaction method was used in the iMLDR. For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were further distinguished by different extended lengths at the 3’ end. In genotyping, two negative controls were set: one with double-distilled water as template and the other with DNA sample without primers while keeping all other conditions the same in one plate. In addition, a random sample accounting for 10% of the total samples was selected for a secondary genotyping, and the coincidence rate of quality control samples was 100%. The raw data of genotyping were analyzed with ABI 3730XL genetic analyzer (Applied Biosystems) and GeneMapper Software v4.1 (Applied Biosystems).

2.3 | Statistical analysis

The present study used SPSS 23.0 (Armonk, NY: IBM Corp, USA) to perform all statistical analysis, and p < 0.05 was considered as...
the threshold of statistical significance. Healthy control was tested for conformity to Hardy-Weinberg equilibrium with Chi-square. The genotype, allele frequencies differences of FCN1 and FCN3 genes of two groups were compared by logistic regression analyses. The associations between SNPs and PTB risk in two genetic models (dominant, recessive model) were also analyzed, and haplotype analysis was conducted by using SHEsis software. We also explored the relationship between FCN1 and FCN3 genes variation and multiple clinical manifestations in PTB patients by Chi-square test or Fisher exact tests.

3 | RESULTS

A total of 317 males and 172 females with a mean age of 44.94 ± 17.78 years were recruited in PTB group. In addition, control group consisted of 224 males and 265 females, with an average age of 43.41 ± 12.96 years. Among 489 PTB patients, the common complications and clinical characteristics of PTB patients were pulmonary infection (105, 21.47%), fever (86, 17.78%), drug resistance (75, 15.34%), DILI (70, 14.31%), hypoproteinemia (34, 6.95%), and leukopenia (33, 6.75%) (Table 1).

The genotype distributions of all SNPs in healthy controls were in accordance with Hardy-Weinberg equilibrium (rs10120023, p = 0.051; rs1071583, p = 0.232; rs3813800, p = 0.763; rs10794501, p = 0.219).

3.1 | Association of FCN1 and FCN3 genes variation with PTB

The allele and genotype frequencies of rs10120023, rs1071583, rs3813800, and rs10794501 were summarized in Table 2. We found that the allele frequencies of FCN1 rs10120023 and rs1071583 were similar among PTB patients and controls, and there was no significant difference in genotype distributions of FCN1 rs10120023 and rs1071583 between PTB patients and controls. Moreover, no significant association between FCN1 rs10120023 and rs1071583 and the risk of PTB was found under the dominant, recessive modes.

FCN3 rs3813800 allele, genotype distribution was not significantly different among PTB patients and controls. Similarly, our results showed no significant difference existed in FCN3 rs10794501 allele, genotype distribution among PTB patients and controls. FCN3 rs10794501 polymorphism was also not associated with PTB susceptibility under the dominant, recessive modes.

3.2 | Relationship between FCN1 and FCN3 genes variation and clinical manifestations of PTB patients

We further compared the genotype and allele frequencies of these SNPs in PTB patients with and without several clinical features including fever, drug resistance, DILI, pulmonary infection, hypoproteinemia, and leukopenia, respectively (Table 3). In FCN1, the TT genotype frequency of rs1071583 variant was significantly increased in PTB patients with drug resistance when compared to these patients without drug resistance (p = 0.040). In addition, the GG genotype and G allele frequencies of FCN3 rs3813800 variant in PTB patients with pulmonary infection were significantly higher than that in PTB patients without pulmonary infection (p = 0.027, p = 0.020, respectively). However, other differences were not statistically significant.

3.3 | Haplotype analysis

Three main haplotypes (CC, CT, and TC) for FCN1 and three main haplotypes (CT, GA, and GT) for FCN3 were detected SHEsis software in this study, and we compared the differences regarding these haplotype distributions between PTB patients and controls. No significant difference was found (Tables 4-5).

4 | DISCUSSION

Innate immune molecules could limit the early stages of infection through a variety of different recognition and effector mechanisms, such as complement system. Studies had confirmed that ficolins and MBL played important roles in the innate immune system by recognizing invading microorganisms and promoting apoptotic cells, and then activating the complement system with the lectin pathway. Since ficolins and MBL were closely related to the MTB infection process, the roles of ficolins, MBL, especially in terms of genetic variation, in the pathogenesis of PTB had also attracted a lot of attention. A new meta-analysis suggested that the rs1800450 and rs5030737 variation in MBL-2 gene as risk factors for PTB, and the rs1800451 and rs7095891 variation in MBL-2 gene were protective

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**TABLE 1** The main demographic and clinical characteristics of study subjects

| Characteristics          | PTB (n = 489) | Controls (n = 489) |
|--------------------------|--------------|--------------------|
| Demographic characteristics |              |                    |
| Age (years)              | 44.94 ± 17.78 | 43.41 ± 12.96       |
| Sex (male/female)        | 317/172      | 224/265             |
| Clinical characteristics |              |                    |
| Fever (+/-)              | 86/393       | NA                 |
| Drug resistance (+/-)    | 75/406       | NA                 |
| DILI (+/-)               | 70/413       | NA                 |
| Pulmonary infection (+/-) | 105/378     | NA                 |
| Hypoproteinemia (+/-)    | 34/449       | NA                 |
| Leukopenia (+/-)         | 33/450       | NA                 |

PTB, pulmonary tuberculosis; NA: not applicable; +/-: with/without.

* These data were described as mean ± standard deviation.

* part of the study subjects of data missing.
factors against PTB. 25 Another meta-analysis indicated that MBL2 gene rs1800451 and rs5030737 polymorphisms played significant roles in PTB susceptibility.26 In addition, Xu et al. found that FCN2 gene −557 A > G, −64 A > C and +6424 G > T were correlated with PTB in a Chinese population and might be protective factors for PTB.18 The present study was the first analysis of the role of FCN1 gene rs10120023 and rs1071583 polymorphisms and FCN3 gene rs3813800 and rs10794501 polymorphisms in PTB in a Chinese population.

Because ficolin-1 was secreted by cells crucial for the host response to MTB infection and was thought to play a pivotal role in the first phase of the immune response, many studies had verified the viewpoint that ficolin-1 was involved in the pathogenesis of PTB.15,27 Sokolowska et al. found that the serum ficolin-1

| SNP            | Analyze model | PTB n (%) | Controls n (%) | p value | OR (95% CI) |
|----------------|---------------|-----------|----------------|---------|-------------|
| rs10120023     | Genotypes    | TT        | 9 (1.84)       | 3 (0.61) | 0.104       | 0.34 (0.09,1.25) |
|                |               | CT        | 108 (22.09)    | 117 (23.92) | 0.563      | 1.09 (0.81,1.47) |
|                |               | CC        | 372 (76.07)    | 369 (75.46) | Reference  |             |
| Alleles        | T             | 126 (12.88) | 123 (12.58) | 0.839   | 0.97 (0.75,1.27) |
|                | C             | 852 (87.12) | 855 (87.42) | Reference |             |
| Dominant model | CC            | 372 (76.07) | 369 (75.46) | 0.823   | 0.97 (0.72,1.30) |
|                | CT+TT        | 117 (23.93) | 120 (24.54) | Reference |             |
| Recessive model| TT            | 9 (1.84)   | 3 (0.61)       | 0.097   | 0.33 (0.09,1.22) |
|                | CT+CC        | 480 (98.16) | 486 (99.39) | Reference |             |
| rs1071583      | Genotypes    | TT        | 111 (22.70)    | 103 (21.06) | 0.357  | 0.84 (0.58,1.21) |
|                |               | TC        | 261 (53.37)    | 257 (52.56) | 0.466  | 0.89 (0.66,1.21) |
|                |               | CC        | 117 (23.93)    | 129 (26.38) | Reference |             |
| Alleles        | T             | 483 (49.39) | 463 (47.34) | 0.366   | 0.92 (0.77,1.10) |
|                | C             | 495 (50.61) | 515 (52.66) | Reference |             |
| Dominant model | CC            | 117 (23.93) | 129 (26.38) | 0.377   | 1.14 (0.85,1.52) |
|                | CT+TT        | 372 (76.07) | 360 (73.82) | Reference |             |
| Recessive model| TT            | 111 (22.70) | 103 (21.06) | 0.536   | 0.91 (0.67,1.23) |
|                | CT+CC        | 378 (77.30) | 386 (78.94) | Reference |             |
| rs3813800      | Genotypes    | CC        | 9 (1.84)       | 9 (1.84)  | 0.959  | 0.98 (0.38,2.49) |
|                |               | GC        | 118 (24.13)    | 109 (22.29) | 0.494  | 0.90 (0.67,1.21) |
|                |               | GG        | 362 (74.03)    | 371 (75.87) | Reference |             |
| Alleles        | C             | 136 (13.91) | 127 (12.99) | 0.551   | 0.92 (0.71,1.20) |
|                | G             | 842 (86.09) | 851 (87.01) | Reference |             |
| Dominant model | GG            | 362 (74.03) | 371 (75.87) | 0.507   | 1.10 (0.83,1.47) |
|                | CC+GC        | 127 (25.97) | 118 (24.13) | Reference |             |
| Recessive model| CC            | 9 (1.84)   | 9 (1.84)       | 1.000   | 1.00 (0.39,2.54) |
|                | GC+GG        | 480 (98.16) | 480 (98.16) | Reference |             |
| rs10794501     | Genotypes    | AA        | 4 (0.82)       | 10 (2.04) | 0.125  | 2.49 (0.78,8.02) |
|                |               | AT        | 105 (21.47)    | 98 (20.04) | 0.650  | 0.93 (0.68,1.27) |
|                |               | TT        | 380 (77.71)    | 381 (77.92) | Reference |             |
| Alleles        | A             | 113 (11.55) | 118 (12.07) | 0.726   | 1.05 (0.80,1.38) |
|                | T             | 865 (88.45) | 860 (87.93) | Reference |             |
| Dominant model | TT            | 380 (77.71) | 381 (77.91) | 0.939   | 1.01 (0.75,1.37) |
|                | AA+AT        | 109 (22.29) | 108 (22.09) | Reference |             |
| Recessive model| AA            | 4 (0.82)   | 10 (2.04)      | 0.119   | 2.53 (0.79,8.13) |
|                | TT+AT        | 485 (99.18) | 479 (97.96) | Reference |             |

SNP, single nucleotide polymorphism; PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval; logistic regression analyses were used for statistical analysis.

p < 0.05 was considered as the level of significance.
### TABLE 3  The associations between FCN1 and FCN3 genes polymorphisms and clinical features of PTB patients

| SNP (M/m) | Clinical features | Genotypes n (%) | Alleles n (%) |
|-----------|------------------|-----------------|---------------|
|           |                  | MM             | Mm            | mm            | p value | M     | m     | p value |
| rs10120023 (C/T) | Fever             |                |                |               |         |       |       |         |
| +         |                   | 65 (75.58)     | 19 (22.09)     | 2 (2.33)      | 0.761   | 149 (86.63) | 23 (13.37) | 0.711 |
| -         |                   | 301 (76.59)    | 87 (22.14)     | 5 (1.27)      |         | 689 (87.66) | 97 (12.34)  |       |
| Drug resistance | +                | 54 (72.00)     | 20 (26.67)     | 1 (1.33)      | 0.574   | 128 (85.33) | 22 (14.67)  | 0.376 |
| -         |                   | 314 (77.34)    | 86 (21.18)     | 6 (1.48)      |         | 714 (87.93) | 98 (12.07)  |       |
| DILI      | +                 | 50 (71.43)     | 19 (27.14)     | 1 (1.43)      | 0.552   | 119 (85.00) | 21 (15.00)  | 0.384 |
| -         |                   | 318 (77.00)    | 88 (21.31)     | 7 (1.69)      |         | 724 (87.65) | 102 (12.35) |       |
| Pulmonary infection | +             | 81 (77.14)     | 23 (21.90)     | 1 (0.95)      | 0.810   | 185 (88.10) | 25 (11.90)  | 0.684 |
| -         |                   | 287 (75.93)    | 84 (22.22)     | 7 (1.85)      |         | 658 (87.04) | 98 (12.96)  |       |
| Hypoproteinemia | +              | 24 (70.59)     | 10 (29.41)     | 0             | 0.443   | 58 (85.29)  | 10 (14.71)  | 0.613 |
| -         |                   | 344 (76.61)    | 97 (21.60)     | 8 (1.78)      |         | 785 (87.65) | 113 (12.38) |       |
| Leukopenia | +                 | 24 (72.73)     | 9 (27.27)      | 0             | 0.588   | 57 (86.36)  | 9 (13.64)   | 0.820 |
| -         |                   | 344 (76.44)    | 98 (21.78)     | 8 (1.78)      |         | 786 (87.33) | 114 (12.67) |       |
| rs1071583 (C/T) | Fever            |                |                |               |         |         |       |         |
| +         |                   | 27 (31.40)     | 39 (45.35)     | 20 (23.26)    | 0.136   | 93 (54.07)  | 79 (45.93)  | 0.277 |
| -         |                   | 86 (21.88)     | 217 (55.22)    | 90 (22.90)    |         | 389 (49.49) | 397 (50.51) |       |
| Drug resistance | +               | 23 (30.67)     | 30 (40.00)     | 22 (29.33)    | 0.040   | 76 (50.67)  | 74 (49.33)  | 0.903 |
| -         |                   | 90 (22.17)     | 227 (55.91)    | 89 (21.92)    |         | 407 (50.12) | 405 (49.88) |       |
| DILI      | +                 | 20 (28.57)     | 38 (54.29)     | 12 (17.14)    | 0.353   | 78 (55.71)  | 62 (44.29)  | 0.167 |
| -         |                   | 94 (22.76)     | 220 (53.27)    | 99 (23.97)    |         | 408 (49.39) | 418 (50.61) |       |
| Pulmonary infection | +             | 23 (21.90)     | 56 (53.33)     | 26 (24.76)    | 0.840   | 102 (48.57) | 108 (51.43) | 0.569 |
| -         |                   | 91 (24.07)     | 202 (53.44)    | 85 (22.49)    |         | 384 (50.79) | 372 (49.21) |       |
| Hypoproteinemia | +              | 8 (23.53)      | 20 (58.82)     | 6 (17.65)     | 0.721   | 36 (52.94)  | 32 (47.06)  | 0.653 |
| -         |                   | 106 (23.61)    | 238 (53.01)    | 105 (23.38)   |         | 450 (50.11) | 448 (49.89) |       |
| Leukopenia | +                 | 12 (36.36)     | 12 (36.36)     | 9 (27.27)     | 0.097   | 36 (54.55)  | 30 (45.45)  | 0.476 |
| -         |                   | 102 (22.67)    | 246 (54.67)    | 102 (22.67)   |         | 450 (50.00) | 450 (50.00) |       |

(Continues)
level was higher in TB patients when compared with controls, and ficolin-1 might be considered potential marker for the diagnosis of TB, with a high specificity and sensitivity. On the other hand, FCN1 gene polymorphism in multiple diseases had also been studied. Cruyssen et al. analyzed the possible association of rheumatoid arthritis and FCN1 gene polymorphism, and found FCN1 rs1071583 G frequency was increased in rheumatoid arthritis patients when compared to controls. Similarly, another study indicated that FCN1 rs10120023 polymorphism might contribute to rheumatoid arthritis susceptibility. Boldt et al. observed a significant association of rs10120023 with leprosy, the causative agent of which was another mycobacterium, M. lepra. However, in the present study, we found that FCN1 rs10120023 and rs1071583 variant was not significantly associated with PTB susceptibility. The differences in these results might be due to different genotyping methods, sample size, and sample sources. Ficolin-3 was an oligomeric-structured lectin encoded by FCN3 with an important role in the lectin complement pathway and had anti-microbial activities against bacterial, viral infections, and inhibited opportunistic pathogen. At present, the most research on ficolin-3 focused on autoimmune diseases and cancer. Ficolin-3 deficiency was reported to be associated with an increased risk of systemic lupus erythematosus, and serum ficolin-3 level was identified as a potential prognostic biomarker in esophageal cancer patients. While Pieczarka et al. found no significant association between FCN3

**TABLE 3 (Continued)**

| SNP (M/m) | Clinical features | Genotypes n (%) | Alleles n (%) | p value |
|-----------|------------------|----------------|--------------|---------|
|           |                  | MM   | Mm  | mm  | p value | M   | m  | p value |
| DILI      | +                | 56 (80.00) | 12 (17.14) | 2 (2.86) | 0.281 | 124 (88.57) | 16 (11.43) | 0.347 |
|           | -                | 301 (72.88) | 105 (25.42) | 7 (1.69) | 0.707 | 85.59) | 119 (14.41) |
| Pulmonary infection | + | 88 (83.81) | 15 (14.29) | 2 (1.90) | 0.027 | 191 (90.95) | 19 (9.05) | 0.020 |
|           | -                | 269 (71.16) | 102 (26.98) | 7 (1.85) | 0.640 | 84.66) | 116 (15.34) |
| Hypoproteinemia | + | 25 (73.53) | 9 (26.47) | 0 | 0.684 | 59 (87.76) | 9 (13.24) | 0.855 |
|           | -                | 332 (73.94) | 108 (24.05) | 9 (2.01) | 772 (85.97) | 126 (14.03) |
| Leukopenia | + | 22 (66.67) | 11 (33.33) | 0 | 0.346 | 55 (83.33) | 11 (16.67) | 0.514 |
|           | -                | 335 (74.44) | 106 (23.56) | 9 (2.00) | 776 (86.22) | 124 (13.78) |

| rs10794501 (T/A) | Fever | + | 67 (77.91) | 19 (22.09) | 0 | 0.635 | 153 (88.95) | 19 (11.05) | 0.843 |
|                 | - | 306 (77.86) | 83 (21.12) | 4 (1.02) | 0.095 | 88.42) | 91 (11.58) |
| Drug resistance | + | 58 (77.33) | 16 (21.33) | 1 (1.33) | 0.872 | 132 (88.00) | 18 (12.00) | 0.813 |
|                 | - | 317 (78.08) | 86 (21.18) | 3 (0.74) | 720 (88.67) | 92 (11.33) |
| DILI            | + | 53 (75.71) | 17 (24.29) | 0 | 0.605 | 123 (87.66) | 17 (12.14) | 0.826 |
|                 | - | 322 (77.97) | 87 (21.07) | 4 (0.97) | 731 (88.50) | 95 (11.50) |
| Pulmonary infection | + | 81 (77.14) | 24 (22.86) | 0 | 0.542 | 186 (88.57) | 24 (11.43) | 0.932 |
|                 | - | 294 (77.78) | 80 (21.16) | 4 (1.06) | 668 (88.36) | 88 (11.64) |
| Hypoproteinemia | + | 24 (70.59) | 9 (26.47) | 1 (2.94) | 0.270 | 57 (83.82) | 11 (16.18) | 0.211 |
|                 | - | 351 (78.17) | 95 (21.16) | 3 (0.67) | 797 (88.75) | 101 (11.25) |
| Leukopenia      | + | 24 (72.73) | 9 (27.27) | 0 | 0.623 | 57 (86.36) | 9 (13.64) | 0.591 |
|                 | - | 351 (78.00) | 95 (21.11) | 4 (0.89) | 797 (88.56) | 103 (11.44) |

SNP, single nucleotide polymorphism; PTB, pulmonary tuberculosis; M, major alleles; m, minor alleles; Chi-square test or Fisher exact tests were used for statistical analysis.

*p < 0.05 was considered as the level of significance; Bold Values indicated as *p < 0.05.*
The role of FCN3 in PTB should also be analyzed, and we explored the relationship between FCN3 gene rs3813800 and rs10794501 and PTB risk. The results demonstrated that there was no significant association between rs3813800 and rs10794501 polymorphism and PTB. Analogously, no evidence of association with hypertension was observed for rs3813800 and rs10794501 in another study.21

In the process of the occurrence and treatment of PTB, the patients usually had a variety of complications and clinical manifestations, such as fever, drug resistance, DILI, pulmonary infection, hypoproteinemia, and leukopenia. These clinical characteristics had an impact on the rehabilitation of patients and might affect the choice of treatment. Therefore, many studies had also explored the association between genetic variations in multiple genes and these clinical manifestations in PTB patients.31-34 Wu et al. found that IncRNA-HNF1B-3:1 rs2542670 polymorphism was associated with increased risk of leukopenia, and rs4262994 polymorphism could cause the increased susceptibility of PTB patients to fever.31 The results by Pontual et al. shown that ABCB1 rs1128503 polymorphism could affect the occurrence of anti-TB drug resistance in PTB patients.32 In this study, we found that FCN1 rs1071583 variant TT genotype was significantly correlated with an increased risk of drug resistance in PTB patients. Moreover, the GG genotype and G allele frequencies of FCN3 rs3813800 variant were also associated with increased risk of pulmonary infection in PTB patients. Based on these findings, these SNPs had the potential to be predictors of certain symptoms in PTB patients and thus to help develop more appropriate treatment regimens.

In summary, our study demonstrated that FCN1 and FCN3 polymorphisms were not associated with PTB susceptibility in a Chinese population. While, FCN1 rs1071583 variant was significantly associated with the occurrence of drug resistance, and FCN3 rs3813800 variant was significantly related to pulmonary infection in PTB patients. This implied that FCN1 and FCN3 gene variation might contribute to some specific clinical phenotype of this disease. It was worth noting that several limitations in this study might affect the accuracy of our results. Firstly, the lack of information on environmental factors in this study made it impossible to explore the genetic interaction effect. Secondly, our sample size might be relatively small, thus affecting the power of this study. Future larger sample size studies in different populations are required to elucidate the precise roles of FCN1 and FCN3 genes polymorphisms in PTB.

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**CONFLICT OF INTEREST**
The authors declare that they have no conflict of interest.

**AUTHOR CONTRIBUTIONS**
H-ML and D-CM designed the study. YL and Q-EY conducted the experiment. W-HL performed the statistical analyses. X-NL, D-PS, and X-LZ participated in sample collection. YL and H-ML drafted the manuscript. D-CM contributed to the manuscript revision. All the authors approved the final submitted version.

**DATA AVAILABILITY STATEMENT**
Data available on request from the authors.

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**TABLE 4** Haplotype analysis of FCN1 gene in PTB patients and controls

| Haplotype         | PTB n(%) | Controls n(%) | p value | OR (95% CI) |
|-------------------|----------|---------------|---------|-------------|
| rs10120023-rs1071583 |          |               |         |             |
| CC                | 370.78 (37.9) | 395.48 (40.4) | 0.240   | 0.90 (0.75, 1.08) |
| CT                | 481.22 (49.2) | 459.53 (47.0) | 0.344   | 1.09 (0.91,1.30) |
| TC                | 124.22 (12.7) | 119.52 (12.2) | 0.759   | 1.04 (0.80,1.36) |

PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval; Haplotype analysis was conducted with the SHEsis software; Global \( \chi^2 \) is 1.385, df = 2 (frequency < 0.03 in both control and case has been dropped), Fisher’s \( p \) value is 0.500; \( p < 0.05 \) was considered as the level of significance.

**TABLE 5** Haplotype analysis of FCN3 gene in PTB patients and controls

| Haplotype         | PTB n(%) | Controls n(%) | p value | OR (95% CI) |
|-------------------|----------|---------------|---------|-------------|
| rs3813800-rs10794501 |          |               |         |             |
| CT                | 129.65 (13.3) | 126.90 (13.0) | 0.811   | 1.03 (0.79, 1.34) |
| GA                | 106.65 (10.9) | 117.90 (12.1) | 0.455   | 0.90 (0.68,1.19) |
| GT                | 735.35 (75.2) | 733.10 (75.0) | 0.715   | 1.04 (0.85,1.28) |

PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval; Haplotype analysis was conducted with the SHEsis software; Global \( \chi^2 \) is 0.577, df = 2 (frequency < 0.03 in both control and case has been dropped), Fisher’s \( p \) value is 0.749; \( p < 0.05 \) was considered as the level of significance.
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