Research Article

A Common Variant of ASAP1 Is Associated with Tuberculosis Susceptibility in the Han Chinese Population

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Background. ASAP1 (also known as AMAP1 or DDEF1) encodes an Arf GTPase-activating protein (Arf GAP), a multifunctional scaffold protein that induces hydrolysis of GTP bound to the ADP-ribosylation factor (Arf) family GTP-binding proteins. Reduction of ASAP1 expression in vitro was related to suppression of cell migration and invasiveness. The genetic variant rs4733781 of the ASAP1 gene was revealed as a significant locus for tuberculosis (TB) susceptibility, but the results still need to be validated.

Methods. Blood samples from a total of 1914 active TB and healthy controls (HC) were collected to evaluate rs4733781 and the risk of TB. Meanwhile, a total of 48 noninfected HC, latent TB-infected (LTBI) controls, and active TB were collected to assay ASAP1 expression difference among the three groups. The QuantiFERON-TB Gold In-Tube was adopted to identify noninfected HC and LTBI.

Results. The genetic variant of rs4733781 was found to be significantly associated with TB, and the A allele of rs4733781 (C>A) was 0.38 and 0.43 among TB cases and HC, respectively (P = 0.0035). Meanwhile, the peripheral blood monocyte RNA fold changes for the ASAP1 gene among the 16 HC, 16 LTBI, and 16 active TB were 1.088 ± 0.4919, 2.237 ± 0.6505, and 10.12 ± 10.98 (F = 9.559, P = 0.0003), respectively, and the expression of ASAP1 was increased by 2.06-fold (P < 0.0001) and 9.30-fold (P < 0.0052) for LTBI and active TB, when compared to the HC. Conclusions. Our data indicated that the A allele of rs4733781 for the ASAP1 gene was in association with a decreased risk of TB. But not only that, the overexpression of the ASAP1 gene among LTBI and TB was related to the progression of TB, which further implies that the expression of ASAP1 would be a potential biomarker for LTBI and TB diagnoses.

1. Introduction

According to the recent global tuberculosis (TB) report, TB is the ninth leading cause of death worldwide and the leading cause of a single infectious agent, ranking above HIV/AIDS [1].

Meanwhile, a recent cohort study with large-scale population demonstrated a high latent tuberculosis infection (LTBI) rate of around 20% in China [2]. However, not all of those infected individuals will proceed into TB, and it was estimated that around 10% of the LTBI population will finally develop TB in their whole lifetime [3].

As we know, diabetes [4], receiving anti-TNFα drugs [5], and HIV infection are commonly considered the risk factors for TB [6, 7]. Nevertheless, the previous study has provided important clues that the host genetics was indispensable for the development of TB [8]. Previous studies had reported that genetic variation of cytokines would be involved in the susceptibility to TB, such as interleukin 6 [9], interleukin 10 [10], and interleukin 17 [11]. Meanwhile, genetic polymorphism of the vitamin D receptor was also reported in association with TB [12].

In recent years, the whole genome-wide association studies (GWAS) revealed some significant loci which demonstrated...
high correlation with TB [13, 14]. Meanwhile, the peripheral blood gene expression signatures for distinguishing LTBI and active TB patients would be helpful in predicting the risk of TB and even in monitoring the efficacy of the anti-TB treatment process [15, 16].

ASAP1 (also known as AMAP1 or DDEF1) encodes an Arf GTPase-activating protein (Arf GAP), a multifunctional scaffold protein that induces hydrolysis of GTP bound to the ADP-ribosylation factor (Arf) family GTP-binding proteins [17]. Reduction of ASAP1 expression in vitro was connected with suppression of cell migration and invasiveness [18]. Overexpression of ASAP1 has been associated with metastasis in cancers [19, 20].

The genetic variants of ASAP1 were firstly reported in association with TB susceptibility by Curtis et al. in 2015, and the functional genetic variant rs4733781 was revealed as the most significant locus for TB susceptibility [21]. Thereafter, only two studies conducted by Hu et al. and Miao et al., who had tried to validate this significant finding in Chinese Han population, finally reached negative results [22, 23]. Recently, rs4733781 was found in association with TB in Xinjiang Muslim population again [24]. In order to identify the effect of this genetic variant on TB susceptibility, we conducted this case-control study with large sample size in a Han Chinese population. Moreover, we will evaluate the expression of ASAP1 mRNA in TB cases and the controls to further support the functional role of ASAP1 in the development of TB.

2. Methods

2.1. The Sample for rs4733781 Genotyping. In total, 957 new incident TB cases and 957 healthy controls (HC) were recruited for this case-control study, and all the participants were belonging to Chinese Han ethnicity. All enrolled cases were coming from Danyang County and Nanjing City of Jiangsu Province. Meanwhile, the HC were included (Table 1). Culture-confirmed active TB cases without initiating treatment were enrolled from the Chest Hospital of Nanjing from June to August of 2015, and 16 TB cases were finally included for the ASAP1 expression assay (Table 1). The whole blood was obtained for the ASAP1 expression assay from each individual free of TB infection, with LTBI, and of active TB. All experimental protocols in this study were approved by the Institutional Review Board of Center for Disease Control and Prevention of Jiangsu Province, and written informed consent was obtained from each participant before the study.

2.3. Genomic DNA and mRNA Extractions. Genomic DNA was extracted from the whole blood by proteinase K digestion and followed by classical phenol/chloroform extraction and ethanol precipitation. Genomic DNA was dissolved in TE buffer and diluted to 20 ng/μL for use. Genomic mRNA was extracted from the PBMCs by the TRizol® LS reagent (Ambion®, Carlsbad, CA, USA), and the isolation procedure was referred to the manufacturer’s instructions. The genomic mRNA was dissolved with RNase-free water and diluted to 50 ng/μL for use. The quality of the extracted genomic DNA and mRNA was quantitated spectrophotometrically by the NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Genotyping of rs4733781. rs4733781 was genotyped by the TaqMan® SNP Genotyping Assay. The assay probe was commercially provided by Thermo Fisher Scientific (MA, USA). The assay ID was C__28031183_10. SNP genotyping was performed on the QuantStudio™ Dx Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA). The PCR reaction program was as follows: step 1: 60°C for 30 seconds; step 2: 95°C for 10 minutes; step 3: by 40 cycles, 95°C for 15 seconds and 60°C for 1 minute; and step 4: 60°C for 30 seconds.

2.5. mRNA Reverse Transcription. High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) were used for the whole genomic mRNA reverse transcription. The reverse transcription procedure was conducted according to the instructions provided by the manufacturer. Meanwhile, primers for the reference gene GAPDH were designed to assay the quantity of the cDNAs (forward-GAA ATC CCA TCA CCA TCT TCC AGG, reverse-GAG CCC CAG CCT TCT CCA TG), and the length of the amplified GAPDH gene fragment was 120 base pairs.

2.6. Gene Expression Assay. 2X TaqMan® Universal Master Mix II (Applied Biosystems, Foster City, CA, USA) with a reaction volume of 10 μL was used to quantitate the gene expressions. Each reaction contains 5 μL 2X TaqMan® Universal Master Mix II, 0.5 μL TaqMan® Gene Expression Assay Mix, 3.5 μL RNase-free water, and 1 μL cDNA
Table 1: Basic characteristics of participants free of TB infection, with LTBI, and of active TB for the ASAP1 expression assay.

| Participants        | Sample numbers | Ages             | Gender          | Clinical diagnosis                                                                 |
|---------------------|----------------|------------------|-----------------|-------------------------------------------------------------------------------------|
| Noninfected HC      | 16             | 36 ± 5.94 (29–49)| Male: 3, Female: 13 | X-Ray negative, no symptoms of TB, and no other infectious diseases and history of TB reported |
| Latent TB-infected HC| 16             | 40.75 ± 7.34 (30–56) | Male: 2, Female: 14 | X-Ray negative, no symptoms of TB, and no other infectious diseases and history of TB reported |
| Active TB           | 16             | 41.88 ± 20.60 (16–82) | Male: 10, Female: 6 | Culture confirmed, all with clinical symptoms and X-ray abnormality of TB |

HC: healthy controls; TB: tuberculosis.

template. The TaqMan® Gene Expression Assay MIX ID for ASAP1 genes was Hs00393663_m1. 18S was adopted as the reference gene. The gene expression assay was performed on the QuantStudio™ Dx Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA), and the program was as follows: step 1: 95°C for 10 minutes and step 2: by 40 cycles, 95°C for 10 seconds and 60°C for 1 minute.

2.7. GEO Data Analysis for ASAP1 Expression. Public gene expression microarray data (NCBI GEO, https://www.ncbi.nlm.nih.gov/gds/) were employed to evaluate ASAP1 expression differences among HC, LTBI, and TB. Only peripheral whole blood samples were chosen for the ASAP1 expression assay. Three datasets (GSE19491 [26], GSE37250 [27], and GSE42834 [28]) were selected to evaluate ASAP1 expression differences. The log-transformed fold change values for ASAP1 were extracted for between-group comparison.

2.8. Statistics. The unpaired Student t-test and one-way ANOVA were applied to numerical variables, whereas the differences in categorical variables were tested by the χ² test. Hardy-Weinberg equilibrium (HWE) was assessed by the Pearson χ² test. The strength of associations between genotypes and TB was estimated by odds ratio (OR) and its 95% confidence interval (95% CI) through univariate and multivariate logistic regression analyses adjusted by age and gender. Online software GEO2R was applied to test ASAP1 expression among different groups. The Benjamini and Hochberg (false discovery rate) was adopted for the multiple comparison correction. The value of fold change (FC) was calculated for showing the fluctuation of the gene expression among different groups. P value of less than 0.05 was considered statistically significant. All of the analyses were performed by SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

3. Results

A total of 1914 participants were finally recruited with a 1:1 ratio between the cases and controls to evaluate the association between rs4733781 and the risk of TB. Genotyping was failed in 9 samples (3 in the cases and 6 in the controls), so a total of 1905 samples were used for the analysis. The mean age was 53.15 ± 17.05 years for TB cases and 48.78 ± 17.54 years for the HC (P < 0.01 by the unpaired Student t-test for the two groups). Meanwhile, male cases constituted 71.9% (686/954) of the total TB cases. The QFT test was conducted on the peripheral blood sample for each HC to assay the status of LTBI, and 22.5% (215/951) of the HC were detected as LTBI.

As shown in Table 2, rs4733781, located within the intron of ASAP1, was found in significant association with TB. The heterozygote CA genotype was found in significant association with TB (OR = 0.81, 95% CI 0.66-0.99, P = 0.0373), and the mutant homozygote AA was associated with a decreased risk of TB (OR = 0.68, 95% CI 0.51-0.89, P = 0.0059). The dominant model of rs4733781 (CA+AA vs. CC) was also found in statistically significant association with TB (OR = 0.78, 95% CI 0.64-0.94, P = 0.0086). When TB cases were further compared with IGRA positive controls, the association between homozygote AA and TB was not significant (OR = 0.82, 95% CI 0.52-1.28, P = 0.383). However, the homozygote AA genotype of rs4733781 demonstrated a statistically significant association with TB by an OR of 0.64 among TB cases and IGRA negative controls (95% CI 0.47-0.86, P = 0.0034). The distribution of rs4733781 genotypes among IGRA-positive and IGRA-negative controls demonstrated no difference (Table 3).

Three NCBI GEO datasets, GSE19491, GSE37250, and GSE42834, were chosen to test ASAP1 expression difference among HC, LTBI, and TB, and all the enrolled participants were HIV negative. The whole blood monocyte cells were used to assay ASAP1 expression for those three datasets. For GSE19491, ASAP1 expressions among 36 HC, 69 LTBI, and 61 TB showed significant difference (Figure 1(a), F = 14.51, P < 0.0001). Meanwhile, ASAP1 expression among LTBI was higher than HC (t = 3.382, P = 0.001), and ASAP1 expression among the TB group was significantly higher than LTBI (t = 2.216, P = 0.0285). For GSE37250, only 83 LTBI and 97 active TB were included, and ASAP1 demonstrated a higher expression among the TB group than LTBI (Figure 1(b), t = 4.879, P < 0.0001). The GSE42834 dataset only contained 118 HC and 40 TB, and ASAP1 expressions in TB groups were significantly higher than HC as well (Figure 1(c), t = 10.04, P < 0.0001). We also included 16 HC, 16 LTBI, and 16 active TB in our study to evaluate ASAP1 expressions, and we found that ASAP1 expression was increased from HC to LTBI and much higher in TB (Figure 1(d)). The FC of ASAP1 expression among the HC, LTBI, and TB were 1.088 ± 0.4919, 2.237 ± 0.6505, and
Table 2: The genotype distribution of rs4733781 among tuberculosis cases and controls.

| SNP rs4733781 | Cases n (%) | Controls n (%) | n (%) | *OR (95% CI) | P     | IGRA-positive controls n (%) | OR (95% CI) | P     | IGRA-negative controls n (%) | *OR (95% CI) | P     |
|---------------|-------------|----------------|--------|--------------|-------|-----------------------------|--------------|-------|-----------------------------|--------------|-------|
| CC            | 361 (37.8)  | 304 (32.0)     | 1.00 (reference) | 78 (36.3)  | 1.00 (reference) | 226 (30.7) | 1.00 (reference) |
| CA            | 461 (48.3)  | 483 (50.8)     | 0.81 (0.66-0.99) | 102 (47.4) | 0.98 (0.71-1.35) | 381 (51.8) | 0.76 (0.61-0.95) | 0.0159     |
| AA            | 132 (13.8)  | 164 (17.2)     | 0.68 (0.51-0.89) | 35 (16.2)  | 0.82 (0.52-1.28) | 129 (17.5) | 0.64 (0.47-0.89) | 0.0034     |
| CA+AA         | 593 (62.2)  | 647 (68.0)     | 0.78 (0.64-0.94) | 137 (63.7) | 0.94 (0.69-1.28) | 510 (69.3) | 0.73 (0.60-0.90) | 0.0033     |
| MAF           | 0.38        | 0.43           | 0.0086 |              |       |                            |              |       |                            |              |       |
| HWE           | 0.24        |                |        |              |       |                            |              |       |                            |              |       |

*Adjusted by age and gender. HWE: Hardy-Weinberg equilibrium; IGRA: interferon-gamma release assay.
10.12 \pm 10.98 (F = 9.559, P = 0.0003), respectively, and the expression of ASAPI was increased by 2.06-fold (P < 0.0001) and 9.30-fold (P < 0.0052) for LTBI and active TB, when compared to the HC.

4. Discussion

In this case-control study, genetic variant rs4733781 of the ASAPI gene was evaluated for association with the risk of TB, and rs4733781 was found in significant association with a low risk of TB in the Han Chinese population. The expression of ASAPI was increased among LTBI and much higher among TB patients.

SNP rs4733781, located in the intron region of ASAPI, was first found in association with TB risk by Curtis and colleagues in a Russian population [21]. However, the minor allele of rs4733781 in Curtis’s study was C allele, which was different in our study, and Curtis and colleagues found that the C allele was in association with a decreased risk of TB. Not only that, Curtis and colleagues conducted a validation study in the African population, and they found that the C allele frequency in African population was much lower than in Russian, and the association between C allele and TB risk only reached a marginal significance.

In our study, the minor allele of rs4733781 was A allele, and it was found in significant association with a decreased
risk of TB. Based on the QFT test, the control group was classified into HC and LTBI, the subgroups further evaluated their relationship with TB, and we found that A allele was significantly associated with TB between IGRA-negative controls and TB, except among IGRA-positive controls and TB. A previous study conducted in China explored rs4733781 and TB, among the west Han Chinese and Tibetan populations [23], no significant effect of rs4733781 was found on TB, and the A allele frequencies were all above 50%, which was in inverse compared to our findings. The HapMap Han Chinese Being (HCB) data showed that the A allele of rs4733781 was 0.36, which was similar to our study. Another study conducted by Wang and colleagues in Xinjiang Muslim population found that the A allele was associated with a decreased risk of TB in China [24]. However, the minor frequency of rs4733781 in Xinjiang Muslim population was C allele, rather than A allele, which was different from our study, and this difference might be contributed to the different ethnic background. Nevertheless, Wang’s study demonstrated the same effect of rs4733781 in association with TB. The minor allele of rs4733781 (A/C alleles) was different between African population and Asian population. Both minor allele A of rs4733781 in Chinese Han population and minor allele C of rs4733781 in African population showed significant association with a decreased risk of TB. Thus, we postulated that the two alleles, which represented a marker, would relate to other functional genes that are involved in the pathogenesis of TB susceptibility rather than directly involved in the pathogenesis of TB.

The reduction of ASAP1 suppresses cell migration in vitro, and overexpression of ASAP1 has been associated with metastasis in cancer [20].

Macrophages and dendritic cells (DCs) play an important role in TB pathogenesis, especially in the initial stage after Mycobacterium tuberculosis infection. DCs are important in the adaptive immunity initiation, but Mycobacterium tuberculosis is known to inhibit migration as well as other functions of DCs, as a result of a delayed adaptive immunity after M. tuberculosis infection [29, 30]. Just as Curtis and colleagues reported, the expression of ASAP1 was lower among Mycobacterium bovis BCG-infected DCs when compared to noninfected DCs. It provided evidence to support that Mycobacterium tuberculosis infection delays adaptive immunity, and it seems that lower expression of ASAP1 might be involved in the delayed adaptive immunity.

In our study, we found ASAP1 expression was high among the LTBI group and much higher in TB patients, which indicated that ASAP1 expression was increased after Mycobacterium tuberculosis infection, especially in TB disease status. We assayed ASAP1 expression in PBMCs, which was different from DCs. Curtis and colleagues have not detected ASAP1 expression among lymphocytes and monocytes after the infection of Mycobacterium bovis BCG, except DCs. Thus, based on Curtis’ results, ASAP1 expression might be decreased in the early stage of Mycobacterium bovis BCG infection. However, after DCs migrated to the lymph nodes, and T-cells being activated after adaptive immunity, we postulated that the expression of ASAP1 would be stimulated by T-cell-based immunity during tuberculosis disease progression.

In our study, the expression of ASAP1 was assayed from PBMCs, in which the expression of ASAP1 might be different from the DCs. Nevertheless, according to a recent study by Zak et al., whole blood was collected directly into PAXgene blood RNA tubes for evaluation of the whole blood RNA signature changes from free of Mycobacterium tuberculosis infection to infection status and TB disease, and the whole blood-based signatures would be more feasible in predicting progression to active TB disease [15].

In conclusion, our study evaluated SNP rs4733781 on TB risk in a Han Chinese population, and the minor allele of rs4733781 was significantly associated with a decreased risk of TB; the expression of ASAP1 was increased among LTBI and much higher among TB patients when compared to noninfected HC.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest
All the authors declared no conflict of interests.

Authors’ Contributions
CC wrote the main manuscript text; BX and WL designed the study and scientifically edited the manuscript; and CC, YS, YL, HS, and GL designed and conducted the experiments. CC, QZ, and LZ checked the data and performed the analysis. All the authors reviewed the manuscript.

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References
[1] WHO, Global Tuberculosis Control, World Health Organization Genova, 2017.
[2] L. Gao, W. Lu, L. Bai et al., “Latent tuberculosis infection in rural China: baseline results of a population-based, multicentre, prospective cohort study,” The Lancet Infectious Diseases, vol. 15, no. 3, pp. 310–319, 2015.
[3] N. M. Parrish, J. D. D. Dick, and W. R. Bishai, “Mechanisms of latency in Mycobacterium tuberculosis,” Trends in Microbiology, vol. 6, no. 3, pp. 107–112, 1998.
[4] C. Y. Jeon and M. B. Murray, "Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies," *PLoS Medicine*, vol. 5, no. 7, article e152, 2008.

[5] W. G. Dixon, K. L. Hyrich, K. D. Watson et al., "Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR)," *Annals of the Rheumatic Diseases*, vol. 69, no. 3, pp. 522–528, 2010.

[6] E. Liu, A. Makubi, P. Drain et al., "Tuberculosis incidence rate and risk factors among HIV-infected adults with access to antiretroviral therapy," *AIDS*, vol. 29, no. 11, pp. 1391–1399, 2015.

[7] B. J. Kirenga, W. Ssengooba, C. Muwonge et al., "Tuberculosis risk factors among tuberculosis patients in Kampala, Uganda: implications for tuberculosis control," *BMC Public Health*, vol. 15, no. 1, 2015.

[8] G. W. Comstock, "Tuberculosis in twins: a re-analysis of the Prophit survey," *The American Review of Respiratory Disease*, vol. 117, pp. 621–624, 1978.

[9] H. Wang, C. Pang, N. Zeng, C. Wan, Y. Shen, and F. Wen, "Association between the IL-6 gene polymorphism and tuberculosis risk: a meta-analysis," *Infection and Drug Resistance*, vol. 10, pp. 445–454, 2017.

[10] J. Zhang, Y. Chen, X. B. Nie et al., "Interleukin-10 polymorphisms and tuberculosis susceptibility: a meta-analysis," *The International Journal of Tuberculosis and Lung Disease*, vol. 15, no. 5, pp. 594–601, 2011.

[11] Z. G. Yu, B. Z. Wang, J. Li, Z. L. Ding, and K. Wang, "Association between interleukin-1β genetic polymorphisms and tuberculosis susceptibility: an updated meta-analysis," *The International Journal of Tuberculosis and Lung Disease*, vol. 21, no. 12, pp. 1307–1313, 2017.

[12] C. Chen, Q. Liu, L. Zhu, H. Yang, and W. Lu, "Vitamin D receptor gene polymorphisms on the risk of tuberculosis, a meta-analysis of 29 case-control studies," *PLoS One*, vol. 8, no. 12, article e83843, 2013.

[13] S. Mahasirimongkol, H. Yanai, T. Mushiroda et al., "Genome-wide association studies of tuberculosis in Asians identify distinct at-risk locus for young tuberculosis," *Journal of Human Genetics*, vol. 57, no. 6, pp. 363–367, 2012.

[14] T. Thye, African TB Genetics Consortium, F. O. Vannberg et al., "Genome-wide association analyses identifies a susceptibility locus for tuberculosis on chromosome 18q11.2," *Nature Genetics*, vol. 42, no. 9, pp. 739–741, 2010.

[15] D. E. Zak, A. Penn-Nicholson, T. J. Scriba et al., "A blood RNA signature for tuberculosis disease risk: a prospective cohort study," *Lancet*, vol. 387, no. 10035, pp. 2312–2322, 2016.

[16] N. Satproeodprai, N. Wichukchinda, S. Suphankong et al., "Diagnostic value of blood gene expression signatures in active tuberculosis in Thais: a pilot study," *Genes and Immunity*, vol. 16, no. 4, pp. 253–260, 2015.

[17] Z. Nie and P. A. Randazzo, "Arf GAPs and membrane traffic," *Journal of Cell Science*, vol. 119, no. 7, pp. 1203–1211, 2006.

[18] S. Bharti, H. Inoue, K. Bharti et al., "Src-dependent phosphorylation of ASAP1 regulates podosomes," *Molecular and Cellular Biology*, vol. 27, no. 23, pp. 8271–8283, 2007.

[19] T. Müller, U. Stein, A. Poletti et al., "ASAP1 promotes tumor cell motility and invasiveness, stimulates metastasis formation in vivo, and correlates with poor survival in colorectal cancer patients," *Oncogene*, vol. 29, no. 16, pp. 2393–2403, 2010.

[20] D. Lin, A. Watahiki, J. Bayani et al., "ASAP1, a gene at 8q24, is associated with prostate cancer metastasis," *Cancer Research*, vol. 68, no. 11, pp. 4352–4359, 2008.

[21] J. Curtis, Y. Luo, H. L. Zenner et al., "Susceptibility to tuberculosis is associated with variants in the ASAP1 gene encoding a regulator of dendritic cell migration," *Nature Genetics*, vol. 47, no. 5, pp. 523–527, 2015.

[22] R. Miao, H. Ge, L. Xu et al., "Genetic variants at 18q11.2 and 8q24 identified by genome-wide association studies were not associated with pulmonary tuberculosis risk in Chinese population," *Infection, Genetics and Evolution*, vol. 40, pp. 214–218, 2016.

[23] X. Hu, W. Peng, X. Chen et al., "No significant effect of ASAP1 gene variants on the susceptibility to tuberculosis in Chinese population," *Medicine*, vol. 95, no. 21, article e3703, 2016.

[24] X. Wang, A. Ma, X. Han, A. Litifu, and F. Xue, "ASAP1 gene polymorphisms are associated with susceptibility to tuberculosis in a Chinese Xinjiang Muslim population," *Experimental and Therapeutic Medicine*, vol. 15, no. 4, pp. 3392–3398, 2018.

[25] H. S. Whitchurch, M. Scott, D. W. Connell, B. Donges, and A. Lalvani, "IGRAs—the gateway to T cell based TB diagnosis," *Methods*, vol. 61, no. 1, pp. 52–62, 2013.

[26] M. P. R. Berry, C. M. Graham, F. W. McNab et al., "An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis," *Nature*, vol. 466, no. 7309, pp. 973–977, 2010.

[27] M. Kaforou, V. J. Wright, T. Oni et al., "Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study," *PLoS Medicine*, vol. 10, no. 10, article e1001538, 2013.

[28] C. I. Bloom, C. M. Graham, M. P. R. Berry et al., "Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers," *PLoS One*, vol. 8, no. 8, article e70630, 2013.

[29] L. L. Roberts and C. M. Robinson, "Mycobacterium tuberculosis infection of human dendritic cells decreases integrin expression, adhesion and migration to chemokines," *Immunology*, vol. 141, no. 1, pp. 39–51, 2014.

[30] A. J. Wolf, B. Linas, G. J. Trevejo-Nunez et al., "Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo," *Journal of Immunology*, vol. 179, no. 4, pp. 2509–2519, 2007.