A Functional Promoter Polymorphism of IFITM3 Is Associated with Susceptibility to Pediatric Tuberculosis in Han Chinese Population

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Abstract

A susceptibility locus for tuberculosis, a re-emerging infectious disease throughout the world, was previously discovered to exist on chromosome 11p15. IFITM3 gene encoding for interferon inducible transmembrane protein 3, is located at 11p15. It acts as an effector molecule for interferon-gamma, which is essential for anti-tuberculosis immune response. In order to investigate the association between susceptibility to TB and genetic polymorphisms of the IFITM3 core promoter, a case-control study including 368 TB patients and 794 healthy controls was performed in Han Chinese children in northern China. The rs3888188 polymorphism showed significant association with susceptibility to TB. The rs3888188 G allele, acting recessively, was more frequent in TB patients (95% confidence interval: 1.08–1.56, Bonferroni P-value: 0.039). We further assessed the effect of rs3888188 polymorphism on IFITM3 transcription in vitro. As based on luciferase promoter assays, the promoter activity of haplotypes with rs3888188 G allele was lower than that of haplotypes with rs3888188 T allele. Moreover, peripheral-blood mononuclear cells carrying rs3888188 GG genotype showed a reduced IFITM3 mRNA level compared to cells carrying TT or GT genotype. In conclusion, rs3888188, a functional promoter polymorphism of IFITM3, was identified to influence the risk for pediatric TB in Han Chinese population.

Introduction

Tuberculosis (TB), with Mycobacterium tuberculosis (M. tuberculosis) as the major pathogen, is still a major threat to worldwide human health. M. tuberculosis infects approximately one-third of the world’s population. However, only about 10% of infected individuals eventually develop TB disease, whilst the majority of humans are naturally resistant to TB, indicating that host genetic factors play an essential role in determining TB susceptibility [1,2]. Genetic variations in an increasing number of genes have been associated with human susceptibility to TB disease [3–6].

In China, the prevalence of TB in adults is 1.04% by WHO report 2012 [7], while the prevalence of TB in children is 0.918% according to a nationwide survey [8]. Compared with adults, children present a special risk group for TB due to rapid progression of a recent infection towards disease [9–12]. Certain pediatric TB reflects Mendelian predispositions, while adult TB seems to be more complex for genetic predisposition [11]. Studying the effects of the candidate susceptibility genes on pediatric TB may aid in the establishment of more efficient prevention of TB spread.

Interferon inducible transmembrane protein 3 (IFITM3) is a double trans-membrane protein that can be upregulated by interferons. High expression of IFITM3 has been detected in periphery leukocytes, HcLa cell lines, and other cells and tissues [13–20]. IFITM3 participates in interferon-triggered processes, such as homotypic cell adhesion, anti-proliferative activities in tumor pathogenesis, and the innate immune response to virus infections [13–19]. Interferon gamma (IFN-γ) is produced and released in response to the presence of pathogens [21], it is secreted as a major cytokine to activate macrophages during M. tuberculosis infection [22]. Mutant mice lacking Ifng have weakened defense against M. tuberculosis [23], and genetic variations in IFNG and its receptor, IFNGR, have been implicated in susceptibility to human TB [24,25]. IFITM3 has been proven to be an important component of IFN-γ signaling pathway, as down-regulation of IFITM3 via siRNA could largely reduce the anti-virus activities conducted by IFN-γ [18]. Thus, IFITM3 is a potential candidate gene for TB susceptibility.

The IFITM3 gene is located on chromosome 11p15. In this regard, it is worth mentioning that Stein et al. previously found in a genome wide scan that one of the TB-linked loci was located in
this chromosome region [26]. The core promoter region of human *IFITM3* is enriched with regulatory elements (such as ISRE elements, the SP1-binding motif, and other regulatory sequences), and these are thought to be critical for its transcription [27–30]. In this current study, we investigated the relationship between *IFITM3* and susceptibility to TB via polymorphism analysis of its core promoter region around transcription start site. To determine whether or not these *IFITM3* polymorphisms are associated with susceptibility to TB, its alleles and genotypes were analyzed in case-control study in Han Chinese pediatric population.

**Materials and Methods**

**Ethics statement**
Clinical investigation had been conducted according to the principles expressed in the Declaration of Helsinki. This research has been approved by the Ethics Committee of Beijing Children’s Hospital. Written informed consent was obtained from the patients or the guardians of the patients that participated in this research.

**Study sample**
All the participants involved in this research were Han ethnicity and originated from Northern China (Beijing city and surrounding provinces). They were vaccinated with BCG at birth, which was confirmed by the presence of a scar on the shoulder and by their vaccination records. The pediatric TB patients (n = 368) were newly diagnosed to be Pulmonary TB (PTB, pathological changes limited to in lung) or Extra-pulmonary TB (EPTB, pathological changes involving other tissues) by at least two experienced pediatricians in Beijing Children’s Hospital according to the pediatric TB clinical diagnosis standard [31–33]. The standard diagnosis of pediatric TB is made when *M. tuberculosis* is isolated. There were 42 bacteriology confirmed TB patients, in addition to their clinical signs/symptoms suggestive of TB, at least one of the following positive features of (1) plus (2) or (1) plus either two of (3)–(5).

- Positive tuberculin PPD skin-test results (using 5 TU).
- Positive clinical response to vaccination records.
- Contact history (family, close contact).
- Positive chest radiography and computed tomography scan.
- Isolation of *M. tuberculosis* from expectorated sputum, induced sputum, nasopharyngeal aspirates, gastric aspirates, or string tests (or other relevant intrathoracic samples) by at least two experienced pediatricians in Beijing Children’s Hospital according to the pediatric TB clinical diagnosis standard [31–33].

**DNA extraction and genotyping.** Genomic DNA was extracted from peripheral leukocytes by using a Genomic DNA Extraction kit (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). The core promoter of *IFITM3* was amplified by PCR using 5′-GAG CCC TGA AGC GGG ACA GTG-3′ and 5′-TGG TGT CCA GCG AAG ACC AGC-3′ primers and genotyped by sequencing using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

**Plasmid constructs.** Forward 5′-TAT ACT GCA CTC GAG TGG TGT CCA GCG AAG ACC AGC-3′ and reverse 5′-TAT ACT GCA CTC GAG TGG TGT CCA GCG AAG ACC AGC-3′ primers were used to amplify the three most common promoter haplotypes of *IFITM3*. Promoter haplotypes were independently inserted between the NheI and Xbol restriction sites of the pGL3-based plasmid (Promega, Madison, WI, USA) to generate the pGL3-IFITM3 plasmids. Site-directed mutagenesis of the -204 G/T (rs3888188) site of the pGL3-IFITM3 plasmids was carried out using a QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) following the manufacturer’s protocol. This process generated three new constructs with the -204 G/T site changed to -204 T/G. These mutations were confirmed by DNA sequencing.

**Cell culture and luciferase assays**
HeLa cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco BRL, USA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml). Each constructed pGL3-IFITM3 plasmid was transfected into HeLa cells with the pRL-SV40 plasmid (Promega, USA) by using Lipofectamine 2000 (Invitrogen, USA). Firefly luciferase and renilla luciferase (both via pRL-SV40 plasmid) activities were sequentially measured by a luminometer 48 h after transfection, with or without IFN-γ treatment (final concentration of 100 pg/ml and 0 h, respectively) over a serial time span (5 h and 20 h), utilizing a Dual-Luciferase reporter assay system (Promega, USA).

Results were expressed as relative light units of firefly luciferase activity over relative light units of renilla luciferase activity. All experiments were performed in triplicate and repeated three times.

**Expression levels of *IFITM3* in individuals with different genotypes**
A lligated gradient density centrifugation method [14] was used for peripheral blood mononuclear cell separation. Cells were resuspended in RPMI medium with 10% fetal bovine serum. Cells (10⁶ in 0.5 ml) were seeded into each well of a 48-well plate and incubated in 5% CO2 at 37°C for 24 h with or without adding IFN-γ (final concentration of 100 pg/ml) over a serial time span (2 h, 8 h, and 24 h). Total RNA was extracted and used for cDNA synthesis. Real-time PCR was performed in an ABI7300 Sequence Detection System (Applied Biosystems, USA) using SYBR Premix Ex Taq II (TaKaRa Bio, Japan). The primers for *IFITM3* were as follows: forward: 5'-ATG AAT CAC ACT GTC TGG CAA ACC TTC T-3' and reverse: 5'-CTA TCC ATG GGC CTTG GAA GAT CAG-3'. Primers for the control (18 S) reaction were: forward 5'-GGGA GGC CCA CCA CCG GAG T-3' and reverse 5'-TGG ACC CCC GGA CAT GAA C-3'.

**Genetic and Statistical analysis**
Hardy-Weinberg equilibrium (HWE), Linkage disequilibrium (LD), allele frequency, genotype distribution, and haplotype analyses were performed using the SHEsis program (http://analysis.bio-x.cn/myAnalysis.php) [34]. Haplotype frequencies were inferred using the derived expectation maximization (EM) algorithm as implemented in the SHEsis software. Power and sample size calculations were performed using Quanto 1.2.4 software (http://hydra.usc.edu/gxe). Statistical analysis was carried out using the Statistical Package for Social Sciences version 13.0 (SPSS, Chicago, IL). Differences between non-contiguous variables, genotype distribution and allele frequency were tested by Fisher’s exact test. Odds Ratios (OR) and 95% confidence intervals (CI) were examined by logistic regression analysis. The student’s t-test was used to compare the promoter activity between haplotypes or *IFITM3* mRNA level between genotypes. All
multiple testing. The frequency of G allele in TB group was observed after an application of the Bonferroni correction for rs7478728, rs71452596 and rs7479267.

Genotyping and genetic analysis

Eight single-nucleotide polymorphisms (SNPs) were genotyped in the 356 base-pair PCR products of the IFITM3 core promoter region around transcription start site: −291 C>T (rs61876247), −242 C>A (rs35409983), −231 C>T (rs28602580), −204 T>G (rs3888188), −186 T>C (rs6598045), −101 C>T (rs7478728), −178 C>A (rs7479267), −175 C>T (rs7479287). All these SNPs were in Hardy-Weinberg equilibrium (P>0.05) both in the TB and control groups. The genotyping results of detected SNPs are summarized in Table 2. Three SNPs had a minor allele frequency (MAF) lower than 5% in both control and TB groups: rs61876247 (3.4% and 2.6%, in TB and control groups, respectively), rs35409983 (1.4% and 0.6% respectively), and rs28602580 (1.8% and 0.6% respectively). Linkage disequilibrium coefficients (|D'|) between all SNP pairs were calculated, and the absolute LD |D'| = 1 and r² = 1) was found among three SNPs, rs6598045 and rs7478728 alone could represent rs71452596 and rs7479267 (in absolute LD), Haplotype analysis was performed for rs3888188, rs6598045 and rs7478728. Accepting the lowest frequency threshold for this haplotype analysis to be 0.05, three haplotypes including GTT, TCT, and TTC were identified (Table 3). The frequency of GTT haplotype (carrying rs3888188 G allele) was significantly higher in TB patients than that in controls (OR: 1.31, 95%CI: 1.09–1.58, P = 0.004), while the frequency of TCT haplotype (carrying rs3888188 T allele) was significantly lower in the TB group than that in controls (OR: 0.71, 95% CI: 0.57–0.99, P = 0.003).

Promoter activities

Three common promoter haplotypes, GTT, TCT, and TCT were amplified from genomic DNA of known homozygous individuals and subcloned into pGL3-basic plasmid construct to generate pGL3-IFITM3 plasmids. Site-directed mutagenesis was then carried out on rs3888188 SNP in each plasmid. Following transfection and subsequent dual luciferase assays, the transcriptional activity of the GTT (carrying rs3888188 G allele) haplotype promoter was significantly lower than that of TCT and TCT (both

| Table 1. Characteristics of study population. |
|---------------------------------------------|
| Characteristic | TB patients | Controls (N = 794) |
|----------------|-------------|------------------|
|                | PTB(N = 151) | EPTB(N = 217)    | Total TB(N = 368) |  |
| Gender, Male/Female | 93/58   | 140/77       | 233/135    | 520/274 |
| Age, Mean years (SD) | 6.2(4.6) | 5.4(4.7)     | 5.8(4.7)   | 6.3(4.0) |
| Age group, years |          |               |            |
| ≤4, N(%)        | 60(39.7)  | 122(56.2)    | 182(49.7)  | 302(38) |
| 4–6, N(%)       | 23(15.2)  | 22(10.1)     | 45(12.2)   | 133(16.7) |
| 7–9, N(%)       | 28(18.5)  | 27(12.4)     | 55(14.9)   | 171(21.5) |
| 10–12, N(%)     | 22(14.6)  | 34(15.7)     | 56(15.2)   | 135(17) |
| 13–17, N(%)     | 18(11.9)  | 22(10.1)     | 40(10.9)   | 53(6.7) |
| Positive Investigations |       |               |            |
| BCG vaccinated  | 151       | 217           | 368        | 794     |
| PPD skin test   | 151       | 217           | 368        | 0       |
| Mtb Culture     | 16        | 26            | 42         | –       |
| Imaging         | 151       | 100           | 251        | –       |
| Contact history | 32        | 82            | 114        | –       |

PTB, pulmonary tuberculosis; EPTB, extra-pulmonary TB; FOB, fiberoptic bronchoscopy observations. doi:10.1371/journal.pone.0067816.t001
carrying rs3888188 G allele) promoter (Fig. 1A). Furthermore, the transcriptional activity of the promoter construct with the TB-susceptible rs3888188 G allele was lower than that of the rs3888188 T allele for each pair of haplotypes (before and after site-directed mutagenesis) (Fig. 1B).

Expression levels of IFITM3 in lymphocytes with different genotype of rs3888188

As a Mendelian recessive trait was accepted for rs3888188 G allele, we further investigated whether rs3888188 GG genotype would influence IFITM3 mRNA expression level. Fifteen healthy individuals (4 with rs3888188 TT genotype, 6 with TG genotype, Table 2. The genotyping results of SNPs in IFITM3 core promoter.

| Position | Rs No. | Allele/ genotype | TB (n,%) | Control (n,%) | $\chi^2$ | P-value | $P$-value OR [95% CI] |
|----------|--------|------------------|----------|--------------|---------|---------|------------------|
| −291 C>T | rs61876247 | T | 25.3,4 | 41.2,6 | 1.33 | 0.271 | 2.171 [1.32, 2.19] |
|           |        | C | 711,96.6 | 1547/97.4 |         |         |                  |
|           |        | TT | 0.0 | 0.0 | 1.25 | 0.264 | 2.114 |
|           |        | TC | 25.6,8 | 41.5,2 |         |         |                  |
|           |        | CC | 343,93.2 | 753/96.8 |         |         |                  |
| −242 C>A | rs35409983 | A | 10.1,4 | 10.0,6 | 3.13 | 0.077 | 0.615 [2.17, 0.90–5.25] |
|           |        | C | 726/98.6 | 1578/99.4 |         |         |                  |
|           |        | AA | 0.0 | 0.0 | 3.15 | 0.755 | 6.043 |
|           |        | AC | 10.2,7 | 10.1,3 |         |         |                  |
|           |        | CC | 358,97.3 | 784,98.7 |         |         |                  |
| −231 C>T | rs28602580 | C | 13.1,8 | 10.0,6 | 6.63 | 0.010 | 0.080 [2.84, 1.24–6.50] |
|           |        | T | 723,98.2 | 1578,99.4 |         |         |                  |
|           |        | CC | 0.0 | 0.0 | 6.70 | 0.010 | 0.078 |
|           |        | CT | 13.3,5 | 10.1,3 |         |         |                  |
|           |        | TT | 355,96.5 | 784,98.7 |         |         |                  |
| −204 T>G | rs3888188 | G | 487,66.2 | 954,60.1 | 7.92 | 0.005 | 0.039 [1.30, 1.08–1.56] |
|           |        | T | 249,33.8 | 634,39.9 |         |         |                  |
|           |        | GG | 166,45.1 | 272,34.3 | 12.87 | 0.002 | 0.013 |
|           |        | GT | 155,42.1 | 410,51.6 |         |         |                  |
|           |        | TT | 47,12.8 | 112,14.1 |         |         |                  |
| −188 T>C | rs6598045 | C | 135,18.3 | 311,19.6 | 0.50 | 0.479 | 3.832 [0.92, 0.74–1.15] |
|           |        | T | 601,81.7 | 1277,80.4 |         |         |                  |
|           |        | CC | 11.0,3 | 24.0,3 | 0.69 | 0.709 | 5.672 |
|           |        | CT | 113,30.7 | 263,33.1 |         |         |                  |
|           |        | TT | 244,66.3 | 507,63.9 |         |         |                  |
| −181 C>T | rs7478728 | T | 607,82.5 | 1236,77.8 | 6.60 | 0.010 | 0.082 [1.34, 1.07–1.68] |
|           |        | C | 129,17.5 | 352,22.2 |         |         |                  |
|           |        | TT | 251,68.2 | 478,60.2 | 6.99 | 0.030 | 0.243 |
|           |        | TC | 105,28.5 | 280,35.3 |         |         |                  |
|           |        | CC | 12,3.3 | 36,4,5 |         |         |                  |
| −178 C>A | rs71452596 | A | 607,82.5 | 1236,77.8 | 6.60 | 0.010 | 0.082 [1.34, 1.07–1.68] |
|           |        | C | 129,17.5 | 352,22.2 |         |         |                  |
|           |        | AA | 251,68.2 | 478,60.2 | 6.99 | 0.030 | 0.243 |
|           |        | AC | 105,28.5 | 280,35.3 |         |         |                  |
|           |        | CC | 12,3.3 | 36,4,5 |         |         |                  |
| −175 C>T | rs7479267 | T | 607,82.5 | 1236,77.8 | 6.60 | 0.010 | 0.082 [1.34, 1.07–1.68] |
|           |        | C | 129,17.5 | 352,22.2 |         |         |                  |
|           |        | TT | 251,68.2 | 478,60.2 | 6.99 | 0.030 | 0.243 |
|           |        | TC | 105,28.5 | 280,35.3 |         |         |                  |
|           |        | CC | 12,3.3 | 36,4,5 |         |         |                  |

Logistic regression analyses were used for calculating Odds Ratios (OR) and 95% confidence intervals (CI). $\chi^2$ and P-value was determined by Fisher’s exact test. $P$-value was corrected by Bonferroni’s approach. *Calculated from the translation start site.

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and 5 with GG genotypes, age 10 ± 3 years) were recruited for this experiment. IFITM3 mRNA analysis was performed in lymphocytes from these 15 individuals. The relative mRNA levels of IFITM3 in all genotypes continued to rise within 2–24 hours after IFN-γ was added. Lymphocytes carrying the rs3888188 GG genotype had significantly lower IFITM3 expression level compared to the cells carrying other genotypes (TT plus TG) (Fig. 2).

Discussion

Children not only have an increased risk of developing progressive disease following exposure, but have a much greater risk of developing disseminated forms of the disease such as tuberculosis meningitis (TBM). Although this could be largely resulted from the immaturity of the immune response, certain pediatric TB reflects Mendelian predispositions, while adult TB seems to be more complex for genetic predisposition [11].

IFITM3 is an important component of the IFN-γ signaling pathway. This has been demonstrated through down-regulation of IFITM3 via siRNA, which reduced IFN-γ antiviral activity by 40–70% [17]. IFN-γ is thought to be essential factor acting against different infectious agents including tuberculosis. Successful immune reaction against Mycobacterium tuberculosis requires strong IFN-γ signaling. As such, IFITM3 is a potential candidate gene for TB susceptibility. Thus, we speculated that IFITM3 might have a clear influence on the occurrence of TB. Until now, no data on the IFITM3 genetic polymorphisms were published for Han Chinese

| Haplotype | TB, N (%) | Control, N (%) | $\chi^2$-value | P-value | OR [95%CI] |
|-----------|-----------|---------------|----------------|---------|------------|
| GTT       | 466.47(63.4%) | 889.38(56%)  | 8.15           | 0.004*  | 1.31[1.09–1.58] |
| TCT       | 124.95(17%)   | 345.86(21.8%) | 8.71           | 0.003*  | 0.71[0.57–0.89]  |
| TTC       | 113.07(15.4%) | 250.20(15.8%) | 0.22           | 0.641   | 0.94[0.74–1.20] |

*P-value <0.05 indicates a significant difference between TB patients and controls.

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Figure 1. Promoter activity and gene expression analysis of IFITM3. Data are shown as the mean of triplicate determinations ± standard deviation (SD) and are representative of three independent experiments. Significant difference was defined as a P-value <0.05 by two-tailed unpaired t test. (A) Dual luciferase assays for haplotypes composed of rs3888188, rs6598045, and rs7478728: comparison of the relative luciferase activities of the ‘GTT,’ ‘TTC,’ and ‘TCT’ haplotypes; † indicates significant difference in comparisons of both GTT vs. TTC and CTT vs. TCT. (B) Dual luciferase assays for haplotypes before and after site-directed mutagenesis: $\dagger$ indicates a significant difference for GTT vs. TTT, § for GTC vs. TTC, and # for GCT vs. TCT. doi:10.1371/journal.pone.0067816.g001
rs3888188: Data are shown as the mean determinations of IFITM3 expression levels of TB patients than in controls. Since the haplotype (rs3888188 T allele) was significantly lower in TB haplotype (that contains rs3888188 G allele) was significantly inheritance was found significant. Besides, the frequency of GTT group after Bonferroni correction and a recessive model of allele in TB group was significantly higher than that in the control under the sample size used. The frequency of the rs3888188 G frequency and the size of its effect [34]. In our study, a power to a sample set to detect an association greatly depends on the allele linked to the increased risk of TB. In genetic studies, the power of linkage analysis of disease loci and quantitative traits is lower than that for association studies [35].

population. As promoter variation might influence gene expression, we initially investigated the association of polymorphisms in the core promoter region of IFITM3 with TB in a subset of the pediatric population of Han Chinese ethnicity.

This case-control study showed the rs3888188G allele to be linked to the increased risk of TB. In genetic studies, the power of a sample set to detect an association greatly depends on the allele frequency and the size of its effect [34]. In our study, a power to detect an effect for rs3888188 was calculated to be high (99.6%) under the sample size used. The frequency of the rs3888188 G allele in TB group was significantly higher than that in the control group after Bonferroni correction and a recessive model of inheritance was found significant. Besides, the frequency of GTT haplotype (that contains rs3888188 G allele) was significantly higher in TB patients than in controls while the frequency of TCT haplotype (rs3888188 T allele) was significantly lower in TB patients than in controls. Since the IFITM3 gene is located at 11p13.5, these findings may also support the identification of the TB-susceptibility locus at 11p15 suggested by the genome scan [26].

Because IFITM3 plays a pivotal role in IFN-γ signaling and successful immunity against M. tuberculosis requires strong IFN-γ signaling, we further hypothesized that the TB-susceptible rs3888188 G allele could lead to reduced IFITM3 transcription. By dual-luciferase reporter analysis, we found the transcriptional activities of the rs3888188 G allele promoter to be lower than that of the T allele promoter. We also noticed that lymphocytes carrying the rs3888188 GG genotype had significantly lower IFITM3 mRNA level compared with cells carrying other two genotypes (TT plus TG). These observations indicated that insufficient transcriptional activity of IFITM3 could be a risk factor for TB susceptibility and that IFITM3 has a protective role against TB. However, further studies are needed to determine the specific transcription factors involved in the rs3888188-regulated promoter activity.

One possible mechanism by which IFITM3 expression levels may modulate susceptibility to TB is a reduced capacity to eliminate M. tuberculosis in phagolysosomes. IFITM3 participates in the lysosomal pathway and was shown to limit viral replication at a late stage of endocytosis [16], as IFITM3 overexpression lead to the expansion of enlarged acidified compartments consisting of lysosomes. Previously, lysosome related genes, such as LCN1 (encoding solute carrier family 11 member 1, a lysosome membrane protein) [36] and IRGM (encoding immunity-related GTPase family M, mediating fusion of M. tuberculosis-contained endosome with lysosome) [37], were proven to be associated with TB susceptibility. Lysosomal pathway plays an important role in TB pathogenesis [36,37], it may directly determine the successful elimination of M. tuberculosis by macrophages or the survival of M. tuberculosis within macrophages. As sufficient expression of IFITM3 effectively restricted infection by influenza virus [16,17], vesicular stomatitis virus [10], filoviruses, and SARS coronavirus [16], sufficient expression of IFITM3 might also be critical in reducing survival of M. tuberculosis.

The present study was conducted among newly diagnosed pediatric TB patients of Han Chinese origin and healthy controls with the same ethnic background. Association linking IFITM3 to pediatric TB is reported here for the first time and has not been previously investigated in any population.

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Author Contributions

Conceived and designed the experiments: CS AS. Performed the experiments: CS XF FL WJ LS WF QY. Analyzed the data: CS XW. Contributed reagents/materials/analysis tools: CS XW JX QM CZ YG. Experiments: CS XW FL WJ LS WF QY. Analyzed the data: CS XW. Conceived and designed the experiments: CS AS. Performed the

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