Immunology Letters 213 (2019) 62–69

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Positive association between MIC gene polymorphism and tuberculosis in Chinese population

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ARTICLE INFO

Keywords:
MIC
Gene polymorphism
Tuberculosis
Disease susceptibility

ABSTRACT

The disease progression and morbidity of tuberculosis (TB) infections are determined by virulence of the microorganism, host genetic factors and environmental factors. The highly polymorphic MHC class I chain-related gene (MIC) could serve as a potential host genetic candidate. To investigate the association of MIC polymorphism with TB infection, 124 patients and 191 ethnically matched controls from Hunan province, Southern China, were genotyped for the MIC polymorphism using polymerase chain reaction-sequence specific priming and sequencing-based typing. The results showed that allele frequencies of MIC-sequence and MICA-STR were different in TB patients in comparison to normal controls (both $P < 0.05$). MICA-A4 and MICA*012:01 alleles were positive associated (OR = 2.42, 95% CI: 1.69–3.87; OR = 3.41, 95% CI: 2.19–5.33, respectively, both $P_c < 0.05$) while MICA -A5 were inversely associated (OR = 0.59, 95%CI: 0.41–0.94, $P_c < 0.05$) with TB. Homozygote MICA*012:01/012:01 was observed to have significant risk effects on TB (OR = 4.76, 95% CI: 1.94–11.69, P_c0000-0001-5151-1853 < 0.05). Additionally, MICB*008 allele conduct a significant risk effect for TB (OR = 3.17, 95%CI: 1.80–5.61, $P_c < 0.05$). All the data showed that MIC polymorphism was associated with the variable susceptibility to TB in Chinese population.

1. Background

Tuberculosis (TB) remains a major global public health issue nowadays, as an estimated 9.6 million people developed TB and 1.5 million died from the disease in 2014 [1]. Clinical, genetic, epidemiological and immunogenetic studies have demonstrated that tuberculosis (TB) is a multifactorial disease. Its development and natural history result from the interaction of exogenous, environmental factors; however, its manifestation depends on the genetic predisposition of the host. Several studies have found evidence that host genetic factors, particularly major histocompatibility complex (MHC) products, play a crucial role in determining susceptibility to diseases [2–4], but no strong evidence for a single allele associated with susceptibility to TB has found.

As a non-classic HLA gene, major histocompatibility complex class I chain-related gene (MIC) is highly polymorphic. MIC gene family includes seven members (MICA to MICG). Among the gene members, MICA and MICB are functional genes while the rest are pseudogenes [5,6]. The gene product of MICA and MICB is cell surface glycoprotein that can bind to NDG2D, a receptor on natural killer cells, NKT cells γδT cells, or CD8 + T cells. The variation of the amino acid residue in MICA and consequent structural alternation could change its binding affinity to NKG2D [7]. The expression of MICA and MICB are also inducible in fibroblasts, keratinocytes, endothelial cells, etc [8]. Therefore, the binding of MICA and MICB to its cognate receptor, NKG2D, might be the trigger of immune responses to infection. The genetic and structural allelic variability of MICA and MICB molecules may affect the interaction with receptors and thus the effector function of defense cells. Some studies have proved the association of MICA polymorphisms with infection disease, such as inflammatory rheumatic diseases [9]and *Chlamydia trachomatis infections*[10].Moreover, one of the most relevant cytokines involved in the response against tuberculosis is interferon-γ (IFN-γ) [11]. An important source of IFN-γ is from NK cells. Stimulation of NK cells by the NKG2D receptor by its ligands, MICA and MICB, results in the release of the cytokine IFN-γ [12]. We therefore hypothesize that the relationship between polymorphism of the MICA and MICB ligand and susceptibility to tuberculosis infection deserves further study.

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https://doi.org/10.1016/j.imlet.2019.07.008
Received 28 March 2019; Received in revised form 29 July 2019; Accepted 30 July 2019
Available online 07 August 2019

At present, there was no study on the role of MICA and MICB polymorphisms in the susceptibility to the TB infection. We performed a case–control study to assess a genetic association of major histocompatibility complex class I chain-related genes with susceptibility to TB in a sample of patients with pulmonary tuberculosis (PTB) of Hunan province, China.

2. Materials and methods

2.1. Subjects

A total of 124 randomly selected patients (age: from 18 to 92 years old) who were treated for active TB at the Hunan Chest Hospital (Changsha, China) from 2014 to 2017 were surveyed consecutively. These included 90 male and 34 female patients The inclusion criteria were: adult patients newly diagnosed with active TB, having evident lesions of TB by simple X-ray, computed tomography, and positive results of sputum smears or cultures for mycobacteria. All admitted patients were treated according to the "Guidelines for the Implementation of China's Tuberculosis Prevention and Control Plan (2008 Edition)", "Clinical Diagnosis and Treatment Guidelines-the tuberculosis sub-volume" and "Guidelines for Chemotherapy of Drug-Resistant Tuberculosis ". In the control group, 191 volunteers without active TB or a history of TB were enrolled (age: from 19 to 75 years old, 130 male and 61 female). Individuals with latent TB (confirmed by physician and Quantiferon test) history were excluded.

Written informed consent was obtained from each patient and volunteer enrolled in this study. All protocols were approved by the Institutional Review Committee of local authorities.

2.2. DNA extraction and MICA genotyping

Genomic DNA was extracted from fresh peripheral blood leukocytes by standard salting-out method. In order to avoid false typing caused by specimen contamination, after obtaining the patient's blood sample, we divided it into two copies for preservation and extracted the DNA separately for subsequent detection and analysis. MICA genotypes were determined using polymerase chain reaction with sequence-specific primers (PCR-SSP) with modifications [13]. Briefly, 95 primers were designed in 84 SSP mixtures for MICA genotyping and all PCR products were analyzed on a 2.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet (UV) illumination. This detected and differentiated 55 MICA-sequence alleles: from *MICA*001* to *MICA*050* (except *MICA*007:01* from *MICA*026*, and *MICA*002:01* from *MICA*020*). To detect the MICA deletion allele, we applied two PCR primers as described previously [14]. The corresponding MICA-STR alleles can be inquired on the Anthony Nolan Trust (HLA Informatics Group website: www.anthonynolan.com/HIG/seq/nuc/text/mica-nt.txt/), after the sequence alleles were typed. Considering there is still some unreported STR information about the known sequence alleles and there may be some rare alleles that cannot be typed by the PCR-SSP system, we applied sequence-based typing (PCR-SBT) as authentication method to validate the reliability of PCR-SSP [15]. A total of 51 samples was genotyped by PCR-SBT, including some randomly selected samples and all *MICA*00701/026* and *MICA*00201/020* alleles typed by PCR-SSP.

2.3. MICB typing

To maximize throughput for the analysis of large sample sets, we therefore used a system that gave the most genotype information while minimizing the amount of sequencing and the cost. For this reason, we limited ourselves to the information that could be captured with a single pair of external primers. In both genes, exon 1 is ˜8.5 kb from exon 2, exons 2–5 are in close proximity to each other in an approximately 2 kb segment, and exon 6 is ˜2.25 kb centromeric to exon 5. Our MICB primers amplified a 2.1-kb fragment containing exons 2, 3, 4, and 5. PCR was done using MICB primers GGACAGCAGACCTGTGTGTTA (forward) and AAAGGAGCTTTCCCATCTCC (reverse); cycling temperature profiles were adapted as previously described with the following minor modifications [16]. Briefly, MICB sequencing primers: GGACAGCAGACCTGTGTGTTA and TGCATCCATAGCACAGGG for exon 2 and 3; CAGGAGTCCACCCTTGACAT and AAAGGAGCTTTCCCATC TCC for exons 4 and 5. All the PCR reactions were carried out in a 20-μl reaction system, containing 40 ng genomic DNA template, 1 × PCR buffer, 1.5 mmol/L MgCl2, 200 μmol/L dNTP, 0.6 units of Taq polymerase (MBI Fermentas, Lithuania), 0.1 μmol/L of each primers. PCR was carried out in an Eppendorf Mastercycler 5333 thermocycler (Germany) programmed with the following series of thermal cycling conditions: initial denaturation at 95 °C for 5 min; 19 cycles of denaturation at 94 °C for 45 s, annealing at 65 °C for 45 s (reduce by −0.5□C per cycle), and elongation at 72 °C for 2 min; 13 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and elongation at 72 °C for 30 s; and a final extension at 72 °C for 10 min before rapid cooling to 4 °C. The amplified productions were sequenced with sequencing primers and the BigDye Terminator v3.1 Cycle Sequence kit (Axygen Biosicences, Union City, CA, USA) on an ABI 3730XL DNA Analyzer. The sequencing was repeated at least twice with independent PCR for samples showing unexpected association with MICB. The sequence chromatograms were analyzed using program Chromas Lite 2.01 (www.technelysium.com.au/chromas_lite.html). Raw sequencing data were manually reviewed and the haploptypic phase of single nucleotide polymorphisms in each exonic segment was determined on the basis of MICB gene sequence database (http://hla.alleles.org/data/txt/micb_nuc.txt), blast analysis was then performed for DNA sequences against the EMBL Release database. MICB allele was assigned on the basis of the integrated information of exons 2–5.

2.4. Statistical analysis

The allele frequencies of MICA and MICB were estimated by direct counting. Hardy–Weinberg equilibrium (HWE) and haplotypic analysis were performed using the SHESIS software (http://analysis.bio-x.cn/SHEsisMain.htm) [17]. The classic coefficients of LD (Δ) and normalized LD (Δrel) were computed for each individual haplotype as described previously [18]. Fisher's exact test was performed to determine the significance of Δ. The LD analysis was restricted to MICA-MICB haplotypes with observed frequencies > 2.0%. Statistical significance was defined at the 5% level after the p value was adjusted using Bonferroni's multiple correction that is by multiplying the p value by the number of independent comparisons performed.

3. Results

3.1. Demographic information of the study participants

In this study, 124 patients diagnosed with TB and 191 control individuals without history of TB infection were enrolled. Male sex was more prevalent in the case group. The average ages of the case and control group were found to have no significant differences. The genotypes of control were in HWE ($P > 0.05$).

3.2. Association of MICA alleles with TB

A total of 9 MICA-sequence and 5 MICA-STR alleles were determined. As shown in Table 1, the distribution frequency of *MICA*012:01* allele was significantly higher in TB patients than in control subjects, suggesting a risk allele (26.2% vs 9.4%, OR = 3.41, 95% CI: 2.19–5.33, $P_c < 0.0001$).

Among the MICA-STR alleles, the frequency of MICA-A4 was significantly higher in TB patients than in control subjects, which may be a susceptibility gene for TB (37.9% vs 20.1%, OR = 2.42, 95% CI:

Table 1
MICA-sequence and MICA-STR allele frequencies in TB patients and controls.

MICA allele	TB		Control		OR	95%CI	χ^2	*P*	*Pc*
	Number 2n = 248	Frequency (%)	Number 2n = 382	Frequency (%)					
MICA- sequence									
*MICA*002:01	43	17.3	78	20.5	0.82	0.54-1.23	0.92	0.3376	—
*MICA*005	1	0.4	0	0	—	—	—	—	—
*MICA*008:01*	52	21.0	95	24.9	0.80	0.55-1.18	1.28	0.2580	—
*MICA*009:01	2	0.8	0	0	—	—	—	—	—
*MICA*010	1	0.4	21	5.5	0.07	0.01-0.52	11.58	0.007	—
*MICA*012:01	65	26.2	36	9.4	3.41	2.19-5.33	31.47	0.0000	0.0000*
*MICA*017	0	0	3	0.8	—	—	—	—	—
*MICA*019	56	22.6	108	28.3	0.74	0.51-1.07	2.53	0.1117	—
*MICA*045	28	11.3	41	10.6	1.06	0.64-2.07	0.05	0.8268	—
MICA-STR									
MICA-A4	94	37.9	77	20.1	2.42	1.69-3.87	23.95	0.0000	0.0000*
MICA-A5	57	23.0	129	33.8	0.59	0.41-0.94	8.41	0.0037	0.0075*
MICA-A5.1	52	21.0	95	24.9	0.80	0.55-1.33	1.28	0.2580	—
MICA-A6	2	0.8	0	0	0	0	—	—	—
MICA-A9	43	17.3	81	21.2	0.78	0.52-1.34	1.42	0.2332	—

*significant difference ($P < 0.05$).

1.69–3.87, $P_c = 0.0000$) while the MICA -A5 allele was lower, indicating a protective role (23.0% vs 33.8%, OR = 0.59, 95% CI: 0.41–0.94, $P_c = 0.0075$).

3.3. Association of MICA genotypes with TB

As shown in Table2, the distribution frequency of MICA*012:01 homozygous in the TB group was significantly higher than that in the control group, which may be the susceptibility genotypes for TB (15.3% vs 3.7%, OR = 4.76, 95% CI:1.94–11.69, $P_c = 0.0052$).

3.4. Association of MICB alleles with TB

A total of 12 MICB alleles were detected in TB cases. As shown in Table 3, the distribution frequencies of *MICB*008* allele was significantly higher in TB patients than in controls, which suggested that *MICB*008* may be the risk allele for TB (OR = 3.17, 95%CI: 1.80–5.61, $P_c = 0.0005$).

3.5. Association of MICB genotypes with TB

As shown in Table 4, no significant difference was identified about the distribution frequencies of MICB genotypes between the TB group and the controls.

3.6. LD analysis on MICA-MICB haplotypes

As shown in Table 5, 12 MICA-MICB haplotypes with a

Table 2
MICA genotype frequencies in tuberculosis patients and healthy controls.

MICA genotype	TB		Control		OR	95%CI	χ^2	P	Pc
	Number (n = 124)	Frequency(%)	number(n = 191)	Frequency(%)					
MICA*002:01/002 : 01	6	4.8	13	6.8	0.70	0.26–1.88	0.51	0.4736	—
MICA*002:01/005	1	0.8	0	0	—	—	—	—	—
MICA*002:01/008:01	9	7.3	19	9.9	0.71	0.31–1.62	0.67	0.4125	—
MICA*002:01/012:01	9	7.3	2	1	7.40	1.57–34.83	8.61	0.0034	—
MICA*045/008:01	0	0	1	0.50	—	—	—	—	—
MICA*008:01/008:01	5	4	19	9.9	0.38	0.14–1.05	3.74	0.0532	—
MICA*008:01/012:01	7	5.6	7	3.2	1.57	0.54–4.60	0.69	0.4047	—
MICA*009/008:01	1	0.8	0	0.00	—	—	—	—	—
MICA*009/019	1	0.8	0	0.00	—	—	—	—	—
MICA*010/002:01	0	0	6	3.10	—	—	—	—	—
MICA*010/008:01	0	0	3	1.60	—	—	—	—	—
MICA*010/010	0	0	5	2.6	—	—	—	—	—
MICA*010/012:01	1	0.8	0	0.00	—	—	—	—	—
MICA*010/017	0	0	1	0.50	—	—	—	—	—
MICA*010/045	0	0	1	0.50	—	—	—	—	—
MICA*012:01/012:01	19	15.3	7	3.7	4.76	1.94–11.69	13.49	0.0002	0.0052*
MICA*017/017	0	0	1	0.50	—	—	—	—	—
MICA*019/002:01	9	7.3	25	13.10	0.52	0.23–1.15	2.65	0.1032	—
MICA*019/008:01	16	12.9	19	9.9	1.34	0.66–2.72	0.67	0.4148	—
MICA*019/012	8	6.5	11	5.8	1.13	0.44–2.89	0.06	0.8009	—
MICA*019/019	9	7.3	22	11.5	0.60	0.27–1.35	1.54	0.2149	
MICA*019/045	4	3.2	9	4.7	0.67	0.20–2.24	0.42	0.5171	—
MICA*045/002:01	3	2.4	0	0	—	—	—	—	—
MICA*045/008:01	9	7.3	8	4.2	1.79	0.67–4.77	1.39	0.2388	—
MICA*045/012	2	1.6	2	1.0	1.55	0.22–11.14	0.19	0.6613	—
MICA*045/045	5	4.0	10	5.2	0.76	0.25–2.88	0.24	0.6242	—

*significant difference ($P < 0.05$).

Table 3
MICB allele frequencies in patients with and healthy controls.

MICB allele	TB		Control		OR	95%CI	χ^2	P	Pc
	Number(2n = 248)	Frequency (%)	Number (2n = 382)	Frequency (%)					
MICB*005:02	124	50	228	59.7	0.68	0.49-0.93	5.72	0.0168	—
MICB*003	0	0	7	1.8	—	—	—	—	—
MICB*009N	5	2	6	1.6	1.29	0.39-4.27	0.17	0.6766	—
MICB*002:01	31	12.5	70	18.3	0.64	0.40-1.01	3.79	0.0516	—
MICB*005:01	1	0.4	1	0.3	1.54	0.10-24.78	0.10	0.7578	—
MICB*005:03	8	3.2	2	0.5	6.33	1.33-30.08	7.03	0.0080	—
MICB*005:04	2	0.8	0	0	—	—	—	—	—
MICB*006	10	4	0	0	—	—	—	—	—
MICB*007	10	4	3	0.8	5.31	1.45-19.48	7.84	0.0051	—
MICB*008	37	14.9	20	5.2	3.17	1.80-5.61	17.14	0.0000	0.0005*
MICB*011	0	0	1	0.3	—	—	—	—	—
MICB*014	15	6	36	9.4	0.62	0.33-1.16	2.30	0.1291	—
MICB*016	1	0.4	2	0.5	0.77	0.07-8.53	0.05	0.8303	—
MICB*024	4	1.6	6	1.6	1.03	0.29-3.68	0.00	0.9670	—

*significant difference ($P < 0.05$).

frequency > 2.0% was observed in TB patients. Among which the haplotypes *MICB*008-MICA*008:01*, *MICB*014-MICA*045*, and *MICB*008-MICA*012:01* showed significant LD in this study ($P < 0.05$).

3.7. Association of MICA-MICB haplotype with TB

There were 19 types of MICA-MICB haplotypes in the patient group and control group with a frequency > 1.5%. Among these haplotypes, MICA*019-MICB*005:02 and MICA*008:01-MICB*005:02 had the highest frequency in the patient group, with frequencies of 14.6% and 10.4%, respectively. MICA*019-MICB*005:02, MICA*008:01-

Table 4
MICB genotype frequencies in TB patients and healthy controls.

MICB genotypes	TB		Control		OR	95%CI	χ^2	*P*	*Pc*
	Number (n = 124)	Frequency(%)	Number (n = 191)	Frequency(%)					
MICB*002:01/002:01	4	3.2	23	12	0.24	0.08-0.72	7.46	0.0063	—
MICB*002:01/003	0	0	1	0.5	—	—	—	—	—
MICB*002:01/005:01	0	0	1	0.5	—	—	—	—	—
MICB*002:01/005:02	20	16.1	17	8.9	1.97	0.99-3.93	3.79	0.0516	—
MICB*002:01/005:03	2	1.6	0	0	—	—	—	—	—
MICB*002:01/008	0	0	1	0.5	—	—	—	—	—
MICB*002:01/009N	1	0.8	2	1	0.77	0.07-8.56	0.05	0.8299	—
MICB*002:01/011	0	0	1	0.5	—	—	—	—	—
MICB*002:01/024	0	0	1	0.5	—	—	—	—	—
MICB*003/003	0	0	2	1	—	—	—	—	—
MICB*003/005:02	0	0	1	0.5	—	—	—	—	—
MICB*003/016	0	0	1	0.5	—	—	—	—	—
MICB*005:01/005:02	1	0.8	0	0	—	—	—	—	—
MICB*005:02/005:02	38	30.6	91	47.6	0.49	0.30-0.78	8.98	0.0027	—
MICB*005:02/005:03	3	2.4	0	0	—	—	—	—	—
MICB*005:02/009N	0	0	1	0.5	—	—	—	—	—
MICB*005:02/014	0	0	4	2.1	—	—	—	—	—
MICB*005:03/005:03	1	0.8	1	0.5	1.54	0.10-24.93	0.10	0.7575	—
MICB*005:04/005:04	1	0.8	0	0	—	—	—	—	—
MICB*006/006	2	1.6	0	0	—	—	—	—	—
MICB*006/008	6	4.8	0	0	—	—	—	—	—
MICB*007/005:02	0	0	1	0.5	—	—	—	—	—
MICB*007/007	3	2.4	1	0.5	4.71	0.48-45.81	2.16	0.1421	—
MICB*007/008	3	2.4	0	0	—	—	—	—	—
MICB*007/009N	1	0.8	0	0	—	—	—	—	—
MICB*008/005:02	17	13.7	11	5.8	2.60	1.17-5.76	5.87	0.0154	—
MICB*008/005:03	1	0.8	0	0	—	—	—	—	—
MICB*008/008	4	3.2	4	2.1	1.56	0.38-6.35	0.39	0.5329	—
MICB*008/014	1	0.8	0	0	—	—	—	—	—
MICB*008/024	1	0.8	0	0	—	—	—	—	—
MICB*009 N/009N	1	0.8	1	0.5	1.54	0.10-24.93	0.10	0.7575	—
MICB*009 N/014	1	0.8	0	0	—	—	—	—	—
MICB*014/005:02	5	4	10	5.2	0.76	0.25-2.28	0.24	0.6242	—
MICB*014/014	4	3.2	11	5.8	0.55	0.17-1.75	1.06	0.3023	—
MICB*016/005:02	1	0.8	0	0	—	—	—	—	—
MICB*016/009N	0	0	1	0.5	—	—	—	—	—
MICB*024/005:02	1	0.8	1	0.5	1.54	0.10-24.93	0.10	0.7575	—
MICB*024/024	1	0.8	2	1	0.77	0.07-8.56	0.05	0.8299	—

Table 5
Linkage disequilibrium (LD) between MICA and MICB in TB patients.

Haplotypes	HF	Δ	Δmax	Δrel	*P*	*Pc*
MICA*002:01/ MICB*005:02	0.088	0.002	0.087	0.017	0.778	—
MICA*002:01/MICB*008	0.032	0.006	0.147	0.042	0.339	—
MICA*008:01/ MICB*002:01	0.05	0.024	0.184	0.129	0.033	—
MICA*008:01/ MICB*005:02	0.099	−0.006	0.105	−0.057	0.811	—
MICA*008:01/MICB*008	0.021	−0.01	0.031	−0.329	0.021	0.036*
MICA*012:01/ MICB*002:01	0.023	−0.01	0.033	−0.298	0.07	—
MICA*012:01/ MICB*005:02	0.104	−0.027	0.131	−0.206	0.242	—
MICA*012:01/MICB*008	0.09	0.051	0.223	0.229	0	0.000*
MICA*019/MICB*002:01	0.033	0.005	0.198	0.024	0.091	—
MICA*019/MICB*005:02	0.146	0.033	0.113	0.292	0.077	—
MICA*045/MICB*005:02	0.055	−0.002	0.057	−0.027	0.599	—
MICA*045/MICB*014	0.031	0.024	0.106	0.228	0	0.000*

*significant difference ($P < 0.05$).

MICB*005:02 and MICA*002:01-MICB*005:02 had the highest frequency in the control group, with frequencies of 20.8%, 14.3%, and 10.5%, respectively (Table 6). The results showed that the MICA*002:01-MICB*002:01 haplotype frequency was lower in the patient group than in the control group ($P_c = 0.0197$), suggested that the haplotype was negatively associated with TB, while the MICA*012:01-MICB*008 haplotype frequency was higher in the patient group than in the control group ($P_c <$ n0.0001), suggested that the haplotype was positively associated with TB.

3.8. sMICA and sMICB among patients and controls

sMICA/B were measured using ELISA Kit (DuoSet dy1599, DuoSet dy1300, R&D) respectively. The average sMICB concentration was higher in the control group than in the patient group (P < 0.0001), sugestting a protective role in TB (Table 7). The correlation coefficient between MICA genotype and sMICA was calculated to be Eta = 0.340, p = 0.045, the correlation coefficient between MICA genotype and sMICA was calculated to be Eta = 0.446, p = 0.001, suggesting that there was a weak correlation between the concentration of sMICA and the MICA genotype, as well as a moderate correlation between the concentration of sMICB and the MICB genotype in the subjects group.

Table 7
The sMICA/B among patients and controls.

Average serum concentration (pg/mL)	TB(n = 124)	Control(n = 191)	Z	*P*
sMICA	11.265	17.865	−1.051	0.293
sMICB	157.377	173.302	−4.942	< 0.0001*

* significant difference ($P < 0.05$).

Table 8
The correlation between MICA genotypes and DRTB/IFN-γ.

MICA genotype	Drug fast		IFN-γ	
	Positive	Negative	Positive	Negative
MICA*00201/00201	2	4	3	3
MICA*00201/00501	0	1	0	1
MICA*00201/00801	7	2	3	6
MICA*00201/01201	3	6	3	6
MICA*00201/019	5	4	4	5
MICA*00201/045	1	2	1	2
MICA*00801/00801	2	3	2	3
MICA*00801/00901	0	1	1	0
MICA*00801/01201	3	4	1	6
MICA*00801/019	8	8	3	13
MICA*00801/045	2	7	4	5
MICA*00901/019	1	0	1	0
MICA*010/01201	0	1	0	1
MICA*01201/01201	5	14	12	7
MICA*01201/019	4	4	1	7
MICA*01201/045	0	2	1	1
MICA*019/019	3	6	1	8
MICA*019/045	0	4	2	2
MICA*045/045	0	5	4	1
Total	46	78	47	77
Spearman's rho	−0.215		0.055	
P (two-tailed)	0.017*		0.546	

* significant difference ($P < 0.05$).

3.9. Drug resistance and IFN-γ production in patients

All patients were tested for drug sensitivity (using GeneXpert combined with Line probe assay) and IFN-γ levels (using the enzyme-linked immunospot assay T-SPOT kit, Oxford, UK). The correlation coefficient between MICA genotype and drug resistance was calculated to be rho = −0.215, p = 0.017, the correlation coefficient between MICB genotype and IFN-γ production was calculated to be rho = 0.266,

Table 6
The correlation between MICA-MICB haplotypes and TB.

Haplotypes	TB (HF)	Control (HF)	χ^2	*P*	P_c
MICA*002:01/MICB*002:01	0.000	0.044	10.77	0.0010	0.0197*
MICA*002:01/MICB*005:02	0.088	0.105	0.19	0.6615	-
MICA*002:01/MICB*008	0.032	0.038	0.11	0.7434	-
MICA*002:01/MICB*014	0.017	0.000	6.57	0.0104	-
MICA*008:01/MICB*002:01	0.050	0.072	1.18	0.2775	-
MICA*008:01/MICB*005:02	0.099	0.143	1.83	0.1767	-
MICA*008:01/MICB*007	0.016	0.003	3.77	0.0521	-
MICA*008:01/MICB*008	0.021	0.007	2.06	0.1509	-
MICA*010/MICB*005:02	0.000	0.032	7.53	0.0061	-
MICA*012:01/MICB*002:01	0.023	0.003	6.84	0.0089	-
MICA*012:01/MICB*005:02	0.104	0.080	1.55	0.2124	-
MICA*012:01/MICB*006	0.016	0.000	6.57	0.0104	-
MICA*012:01/MICB*008	0.090	0.000	37.36	< 0.0001	< 0.0001*
MICA*019/MICB*002:01	0.033	0.040	0.11	0.7434	-
MICA*019/MICB*005:02	0.146	0.208	2.79	0.0948	-
MICA*019/MICB*014	0.000	0.016	3.72	0.0537	-
MICA*045/MICB*002:01	0.018	0.016	0.02	0.8967	-
MICA*045/MICB*005:02	0.055	0.028	3.59	0.0581	-
MICA*045/MICB*014	0.031	0.052	1.12	0.2901	-

* significant difference ($P_c < 0.05$).

Table 9
The correlation between MICB genotypes and DRTB/IFN-γ.

MICB genotype	Drug fast		IFN-γ	
	Positive	Negative	Positive	Negative
MICB*002/002	1	3	1	3
MICB*002/00502	6	14	6	14
MICB*002/00503	1	1	1	1
MICB*002/009N	0	1	0	1
MICB*00501/00502	0	1	0	1
MICB*00502/00502	17	21	8	30
MICB*00502/00503	1	2	2	1
MICB*00502/008	8	9	7	10
MICB*00502/014	1	4	3	2
MICB*00502/016	0	1	1	0
MICB*00502/024	0	1	1	0
MICB*00503/00503	0	1	1	0
MICB*00503/008	0	1	0	1
MICB*00504/00504	0	1	0	1
MICB*006/006	2	0	2	0
MICB*006/008	3	3	3	3
MICB*007/007	1	2	2	1
MICB*007/008	0	3	2	1
MICB*007/009N	1	0	0	1
MICB*008/008	1	3	1	3
MICB*008/014	0	1	1	0
MICB*008/024	1	0	0	1
MICB*009 N/009N	0	1	0	1
MICB*009 N/014	1	0	0	1
MICB*014/014	1	3	4	0
MICB*024/024	0	1	1	0
Total	46	78	47	77
Spearman's rho	0.008		0.262	
P (two-tailed)	0.933		0.003*	

* significant difference ($P < 0.05$).

p = 0.003, both suggesting a weak correlation. No dectectable correlation was found between the drug resistance and the MICB genotype or between the IFN-γ production and the MICA genotype in the subjects group (Tables 8 and 9).

4. Discussion

TB is a complex disease in which the genetic background has been demonstrated to play an important role [19]. In this paper we describe, for the first time, the importance of the MIC gene polymorphism in Chinese TB patients. Identification of MIC gene conferring susceptibility to TB would greatly expand our knowledge on TB development and disease prevention.

We found a significant risk effect for the MIC alleles on TB, including MICA-A4 and *MICA*012:01*. MICA*012, like MICA*A4, is in LD with MICA-129^{Met}, a polymorphism located in the α2 domain of MICA protein that interacts directly with NKG2D receptor with higher affinity [20], and the T helper type 1 (Th1)/Th2 balance could tend towards Th1, with a predominant cellular response [21]. As the Th1 response is predominant in intracellular infection, the risk role of MICA*A4 in TB makes sense. Meta-analysis suggest that the MICA*A5 alleles may be an important protective factor for cancer in Caucasian populations and for Behcet's disease in the Middle East and East Asia [22,23]. Our data also showed a protective effect for MICA-A5 allele on TB.

As the major ligands of human NKG2D, an activating cell surface receptor expressed on NK cells and on some T-cell subsets, MICA/B proteins play important role in immune surveillance and defense systems against infections [24–26]. The function of MICA proteins was greatly predetermined by genetic polymorphism [27]. For example, individuals who were homozygous for MICA*010 had no stable protein accumulation on their cellular membrane, their cells were naked for the MICA protein, and ligands other than MICA should take the function of activation of NKG2D-mediated response [28,29]. Extensive researches have demonstrated associations between the MICA polymorphism and infectious diseases. Zou et al have found that *MICA*008* appeared to be stably expressed on the cell surface while proteins coded by other MICA alleles were down-regulated after HCMV infection [30].

Our study observed for the first time the positive association between MICA gene polymorphism and TB in Chinese population, although Souzal et al have reported that MICA was not associated with the variable susceptibility to TB in Brazil population [31]. Because MIC alleles are known to be in strong linkage disequilibria with alleles of HLA-B as well as HLA-A, -C, DRB1, and DQB1, and it has been reported the association of HLA and MICA genes with tuberculosis in a Brazilian population, which suggest the association between MIC and tuberculosis was a reflection of its strong linkage disequilibrium with HLA. This could also exist in our present findings, so, our future work should include analyzing the different distributions of HLA gene polymorphisms among TB patients and controls, and to confirm the role of MIC gene polymorphism in the mechanism of TB. The polymorphism of MICA gene has been extensively investigated in many different worldwide ethnic populations, and available data indicate great difference of distributions of MICA/B alleles and MICA/B allele-containing haplotypes among populations [32–35]. Our present data showed that the MICA*002:01-MICB*002:01 haplotype frequency was lower in the patient group than in the control group ($P_c = 0.0197$), suggesting that the haplotype might be a protective genetic factor for TB, while the MICA*012:01-MICB*008 haplotype frequency was higher in the patient group than in the control group ($P_c < 0.0001$), suggesting a risk genetic factor for TB. Considering the limited sample size, we need to replicate the present finding in different ethnic groups with larger number of samples. At the protein level, we observed that the average sMICA level in the patient group was lower than that in the control group, but there was no significant difference. Further analysis showed that there was a weak correlation between sMICA level and MICA genotype. Studies have confirmed that certain genotypes, such as MICA*010, are likely to produce more sMICA, which may show a certain degree of superiority in resisting some pathogenic infections. In our study, we also found a weak correlation between MICA genotype and TB drug resistance. At present, we do not know how to explain this phenomenon. We can not exclude that this is a meaningless statistical result due to insufficient sample size, but here is a bold hypothesis that intestinal flora may be involved in the mechanism [36,37].

Reports on the association of MICB with disease were less than that of MICA, which might be due to that MICB was less polymorphic than MICA and was thus considered less informative in terms of evaluating disease association. However, accumulating researches show that MICB polymorphism was related to several tumor, infect diseases and autoimmune diseases [38–42]. To our knowledge, this is the first study reporting association of MICB polymorphism with TB susceptibility. Considering that there is a long sequence of more than 8 kb between Exon 1 and Exon 2 of MICB, and that Exon 1 of MICB encodes a leading peptide chain, which will be hydrolyzed before the formation of the final MICB protein molecule and is not directly involved in the interaction between MICB and NKG2D molecules. Therefore, the polymorphism analysis of Exon 1 of MICB has not been included in our study. We found a significant risk effect for the *MICB*008* allele on TB. The MICB*008 allele, with a reduced promoter activity [43], belongs to an ancestral haplotype (EH 8.1), and a decreased response against viral infections in individuals carrying EH 8.1 haplotype has been reported [44,45]. At the protein level, we observed that the average sMICB level in the patient group was significantly lower than that in the control group, and sMICB level was moderately correlated with MICB genotype, we also found a weak correlation between MICB genotype and IFN-γ production, which is considered crucial for Th1 polarization [46], promoting functions of both NK cells and cytotoxic T lymphocytes. The role of MICB alleles in the natural immune response against intracellular infections should be evaluated, a profound dysregulation of MICB expression may underlie the association between alleles and

disease susceptibility.

Our findings support the concept that the NKG2D/ligand system plays an important role in the immune defense. However, several limitations should be considered in our study. The first one is that we did not discriminate whether the TB patients in our study were HIV (human immunodeficiency virus)-positive. HIV weakens the immune system, thus increases the chance of active TB. The second limitation is that we did not analyze whether patients were resistant to treatment. Drug-resistant TB is caused by inconsistent or partial treatment when patients do not take all their medicines regularly for the required period of time. The third limitation of our study is the relatively small sample size of cohort study, therefore further work needs to be done.

5. Conclusions

Our observations from the Chinese TB patients suggest that different compositions of host MIC alleles probably indicate risk markers for TB. These results are novel and of interest for understanding the genetic basis of TB. Studies with different populations may establish new evidence and further our understanding of TB pathogenesis.

Declaration of Competing Interest

The authors declare that they have no conflict of interests or commercial links pertinent to the manuscript.

Acknowledgement

We are grateful to Mr. Lin Jianlin from the School of Public Health of Xiamen University for his valuable advice on statistical analysis.

Funding

This study were supported by grants from the National Natural Science Foundation of China (project No: 31200141), Youth Foundation of Fujian Educational Committee (project No: JAT170713) and Natural Science Foundation of Fujian Science and Technology Department (project No: 2017D0014).

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