Cytokine 123 (2019) 154775

Contents lists available at ScienceDirect

Cytokine

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

Genetic variants in IFNG and IFNGR1 and tuberculosis susceptibility

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ARTICLE INFO

Keywords:
IFNG
IFNGR1
Tuberculosis
Susceptibility

ABSTRACT

Background: Tuberculosis (TB) is the type of chronic infectious disease which majorly caused by *Mycobacterium tuberculosis* (*M. TB*). Emerging data suggest that interferon gamma (IFNG) and its receptor IFNGR1 may be involved in the risk of TB.

Methods: A total of 636 TB patients and 608 healthy controls were selected. The association between single nucleotide polymorphisms (SNPs) and TB was estimated by logistic analyses adjusting for age, gender and smoking status. SNPs genotyping was done by using the improved multiplex ligase detection reaction (iMLDR).

Results: The *IFNG* rs1861494 allele C was related to an increased risk for TB (OR = 1.25, 95%CI: 1.06–1.48; P = 0.009). Compared with TT genotype, CT (OR = 1.28, 95%CI: 1.01–1.63; P = 0.040) and CC (OR = 1.51, 95%CI: 1.04–2.19; P = 0.031) were also risk factors for TB. In the subgroup analysis, the association was stronger among participants < 25 years (OR = 2.40, 95%CI: 1.70–3.38; P < 0.001) and male groups (OR = 1.31, 95%CI: 1.03–1.66; P = 0.030). In addition, *IFNG* rs1861494 was associated with anti-TB treatment outcome (OR = 0.70, 95%CI: 0.52–0.94; P = 0.017). We also detected that *IFNGR1* rs2234711 influenced the IFNG production.

Conclusion: *IFNG* rs1861494 polymorphism was associated with TB, particularly in the younger and male subgroups.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. TB*), is still the majorly global causes of morbidity and mortality. The World Health Organization (WHO) in 2017 reported that almost 10.4 million TB were diagnosed and 1.7 million died from TB [1]. The one-third population of the world is detected to be latent infection with *M. TB*. However, approximately 5–10% of them will ever develop active TB. TB is a complex disease and it is not fully known why only some infected individual progress to TB but others do not. It was suggested that TB development is not only attributed to environmental factors but also by genetic factors which were proved by Genome-wide association study (GWAS) [2] and twin study [3].

Previous data demonstrated that the interferon gamma (IFNG) is essential to control TB infection [4]. IFNG secreted by natural and T cells is a critical T helper type 1cytokine. The knockout *IFNG* gene mice that were infected with *M. TB* had higher bacilli loads and decreased expression of reactive nitrogen intermediates [5]. Individuals with IFNG-mediated immune hereditary diseases tend to be particularly infected with *M. TB* [6]. IFNG is up-regulated and secreted to activate macrophages during *M. TB* infection [7]. Activated macrophages could increase the production of reactive oxygen and nitrogen intermediates, then resulting in an unfavourable environment for intracellular mycobacteria. Patients with bronchial asthma had a high release of IFNG induced by bronchoalveolar lavage leukocytes [8]. In severe injuries patients with infection, IFNG treatment experienced fewer deaths, indicating that IFNG is necessary to control infection [9]. What's more, previous studies have demonstrated that *IFNG* single nucleotide polymorphisms (SNPs) were associated with susceptibility to TB [10–12]. Also, a recent meta-analysis of 11 studies reported that IFNG + 874T/A allele showed a significantly decreased risk against TB susceptibility [13]. In general, all of the above information suggested that *IFNG* was a causal gene for TB risk.

IFNG exert its function via triggering signaling cascades by binding to two types of receptors including IFNG receptor (IFNGR1 and IFNGR2). IFNG homodimers interact with the two receptors, which result in receptor dimerization [14]. *IFNGR1* located on chromosome 6q23.4 encodes the ligand-binding chain (alpha) of IFNGR. *IFNGR1* has been reported to be associated with numerous diseases such as cerebral malaria [15], Leishmania donovani [16], gastric carcinoma [17] and Helicobacter pylori infection [18]. As a critical molecule of IFNG signaling pathway, IFNGR1 has previously been found to play a critical

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https://doi.org/10.1016/j.cyto.2019.154775
Received 22 May 2018; Received in revised form 6 July 2019; Accepted 8 July 2019
Available online 13 July 2019

Table 1
Demographic distribution of healthy controls and tuberculosis patients.

Parameters	Cases, n = 636	Controls, n = 608	*P* value
Age, (years)*	36.8 ± 15.7	37.1 ± 15.7	0.677
Male, n (%)	324 (50.9)	302 (49.7)	0.654
Smoking, n(%)	195(30.7)	141(23.2)	0.002
Location of TB, n (PTB/EPTB)	276/360		
Acid-fast bacilli stain positive, n (positive/negative)	138/360		
Culture positive n (positive/negative)	32/126		
TB-DNA positive n (positive/negative)	122/133		

Abbreviations: SD, standard deviation; PTB, pulmonary tuberculosis; EPTB, extra-pulmonary tuberculosis.
* Data are presented as mean ± SD.

role in the pathogenesis of TB. Evidence for human genetic components in susceptibility to TB demonstrated that some genetic polymorphisms of cytokine genes, including IFNGR1, were associated with other viral/host-mediated immune responses in TB [19–20]. Moreover, defects in IFNGR1 confer dominant susceptibility to *M. TB* infections. IFNGR1 genetic polymorphisms have been associated with the tuberculosis susceptibility among different populations [21–23].

However, the association of polymorphisms in *IFNG* and *IFNGR1* with TB had not been researched in the west Chinese population. Thus, the present study aimed to investigate the association between tag SNPs of *IFNG* and *IFNGR1* and TB susceptibility in this population.

2. Material and methods

2.1. Cases and controls

TB cases and healthy controls were enrolled from those attending the West China Hospital of Sichuan University. The diagnosis of TB was based on clinical manifestations, smear/culture/TB-DNA positive results, radiography and response to anti-TB treatment, following the guidelines of the WHO [1]. Those participants with HIV, cancer, immune-related diseases and other lung diseases were excluded from this study. None of them was blood relationship. They were followed up at least 18 months, and the treatment outcomes categories were defined according to previous study [24]. All subjects were required to sign the written informed consent for their involvement in the present study. The specialized nurses then obtain 2–5 ml venous blood samples from them. Genomic DNA was extracted from blood samples based on the manufacturer's instructions (Axygen Scientific Inc, Union City, CA, USA). Our study was conducted based on the Helsinki declaration. This study was approved by the Ethics Committees of the West China Hospital of Sichuan University.

2.2. Snps selection and genotyping

Tag SNPs in the IFNG/IFNGR1 were selected for genotyping. The selection of tag SNPs was according to following criteria: 1) minor allele frequency (MAF) ≥0.1 in Chinese Han population; 2) linkage disequilibrium (LD) $r^2 \geq 0.8$; and 3) Hardy-Weinberg equilibrium (HWE) test P-value ≥0.05. SNPs genotyping was done by using the improved multiplex ligase detection reaction (iMLDR) (Genesky Biotechnologies Inc., Shanghai, China). For quality control, we randomly chose 5% subjects to repeat genotype.

2.3. IFNG release assay

A total of 92 active TB patients in this study were detected IFNG release assay QuantiFERON-TB Gold In-Tube (QFT-GIT) according to the manufacturer (Cellestis, Carnegie, Australia). The positive and negative results were obtained based on the cut-off values suggested by the manufacturer.

2.4. Statistical analysis

Differences in the distributions of continuous variables between cases and controls were calculated using student's *t*-test. HWE and gender distributions were assessed by χ^2 –test. The association between SNPs and TB susceptibility was estimated by logistic analyses adjusting for age, gender and smoking status. The haplotypes and level of LD were calculated using SHEsis online software (http://analysis.bio-x.cn). All simulations were done by SPSS version 19 (IBM; Armonk, NY). Scatter plot was done by GraphPad Prism 5.0 (Graph-Pad Software, San Diego, CA, USA). $P < 0.05$ was the cut-off values of statistic differences.

3. Results

3.1. Demographics of the participants

As showed in Table 1, a total of 636 TB patients (mean age, 36.8 ± 15.7 years; 324 males and 312 females) and 608 healthy controls (mean age, 37.1 ± 15.7 years; 302 males and 306 females) were selected. The ethnicity of them was Han Chinese. The distributions of age and gender between cases and controls were well matched ($P > 0.05$). The smoking ratio of cases was higher than that of controls ($P = 0.002$).

3.2. Genotype analyses of the risk of tuberculosis

Two tag SNPs (rs2069718 and rs1861494) within *IFNG* and four tag SNPs (rs3799488, rs9376267, rs1327475 and rs2234711) within *IFNGR1* were identified (Table 2). All these tag SNPs were in HWE in the control group. As showed in Table 3, the *IFNG* rs1861494 was associated with TB susceptibility. The allele C was related to an increased risk for all active TB (OR = 1.25, 95%CI: 1.06–1.48; P = 0.009).

Table 2
Basic information of all SNPs in our study.

Gene/SNPs	chromosome	Location	Functional Consequence	MA	MAF	HWE
IFNG						
rs2069718A > G	12	68,550,162	intron3	G	0.138	0.966
rs1861494T > C	12	68,551,409	intron3	C	0.308	0.863
IFNGR1						
rs3799488T > C	6	137,519,780	intron6	C	0.249	0.983
rs9376267C > T	6	137,531,031	intron1	T	0.452	0.319
rs1327475G > A	6	137,536,455	5′FLANKING	A	0.116	0.706
rs2234711G > A	6	137,540,520	5′UTR_exon1	A	0.407	0.150

Abbreviation: SNP, single nucleotide polymorphism; MA, minor allele; MAF, minor allele frequency; HWE, Hardy Weinberg equilibrium.

Table 3
Genotype distribution of *IFNG* and *IFNGR1* polymorphisms.

Gene/SNPs	Case(%), n = 636	Control(%), n = 608	$P^{\#}$	OR$^{\#}$(95%CI)	IGRA(+), n = 81	IGRA(-)*, n = 11	$P^{\#}$	OR$^{\#}$(95%CI)
IFNG								
rs2069718A > G								
Genotype								
AA	467(73.9)	441(72.9)			57(70.4)	8(72.7)		
GA	156(24.7)	152(25.1)	0.821	0.97(0.75–1.26)	24(29.6)	3(27.3)	0.775	1.23(0.29–5.18)
GG	9(1.4)	12(2.0)	0.407	0.69(0.29–1.66)	0(0)	0(0)	–	
Allele								
A	1090(86.2)	1034(85.5)			138(85.2)	19(86.4)		
G	174(13.8)	176(14.5)	0.575	0.94(0.75–1.18)	24(14.8)	3(13.6)	0.797	1.19(0.32–4.40)
Genetic model								
Dominant			0.694	0.95(0.74–1.22)			0.775	1.23(0.29–5.18)
Recessive			0.429	0.70(0.29–1.68)			–	
rs1861494T > C								
Genotype								
TT	262(41.5)	291(48.3)			35(43.2)	7(63.6)		
CT	288(45.6)	251(41.7)	0.040	1.28(1.01–1.63)	34(42.0)	3(27.3)	0.324	2.08(0.48–8.96)
CC	81(12.8)	60(10.0)	0.031	1.51(1.04–2.19)	12(64.2)	1(9.1)	0.289	3.53(0.34–36.4)
Allele								
T	812(64.3)	833(69.2)			104(64.2)	17(77.3)		
C	450(35.7)	371(30.8)	0.009	1.25(1.06–1.48)	58(35.8)	5(22.7)	0.271	1.83(0.62–5.35)
Genetic model								
Dominant			0.013	1.33(1.06–1.66)			0.249	2.19(0.58–8.27)
Recessive			0.115	1.33(0.93–1.90)			0.644	1.71(0.18–16.80)
IFNGR1								
rs3799488T > C								
Genotype								
TT	354(56.0)	326(53.9)			45(55.6)	7(63.6)		
CT	241(38.1)	235(38.8)	0.651	0.95(0.75–1.20)	32(39.5)	4(36.4)	0.593	1.47(0.36–5.96)
CC	37(5.9)	44(7.3)	0.335	0.80(0.50–1.27)	4(4.9)	0(0)	–	
Allele								
T	949(75.1)	887(73.3)			122(75.3)	18(81.8)		
C	315(24.9)	323(26.7)	0.336	0.92(0.76–1.10)	40(24.7)	4(18.2)	0.398	1.66(0.51–5.36)
Genetic model								
Dominant			0.474	0.92(0.74–1.15)			0.469	1.67(0.42–6.70)
Recessive			0.333	0.80(0.51–1.26)			–	
rs9376267C > T								
Genotype								
CC	182(28.8)	178(29.4)			15(18.5)	3(27.3)		
CT	329(52.1)	283(46.8)	0.330	1.14(0.88–1.48)	52(64.2)	6(54.5)	0.442	1.84(0.39–8.66)
TT	121(19.1)	144(23.8)	0.274	0.84(0.61–1.15)	14(17.3)	2(18.2)	0.573	1.96(0.19–20.29)
Allele								
C	693(54.8)	639(52.8)			82(50.6)	12(54.5)		
T	571(45.2)	571(47.2)	0.332	0.93(0.79–1.08)	80(49.4)	10(45.5)	0.608	1.27(0.51–3.19)
Genetic model								
Dominant			0.791	1.03(0.81–1.32)			0.382	1.96(0.44–8.80)
Recessive			0.050	0.76(0.58–1.00)			0.952	1.05(0.19–5.80)
rs1327475G > A								
Genotype								
GG	486(76.9)	471(77.9)			65(80.2)	8(72.7)		
GA	135(21.4)	128(21.2)	0.876	1.02(0.78–1.34)	15(18.5)	3(27.3)	0.492	0.59(0.13–2.65)
AA	11(1.7)	6(1.0)	0.264	1.77(0.65–4.83)	1(1.2)	0(0)	–	
Allele								
G	1107(87.6)	1070(88.4)			145(89.5)	19(86.4)		
A	157(12.4)	140(11.6)	0.515	1.08(0.85–1.38)	17(10.5)	3(13.6)	0.667	0.74(0.19–2.87)
Genetic model								
Dominant			0.688	1.06(0.81–1.38)			0.559	0.64(0.15–2.84)
Recessive			0.265	1.77(0.65–4.81)			–	
rs2234711G > A								
Genotype								
GG	215(34.0)	224(37.0)			29(35.8)	4(36.4)		
GA	300(47.5)	269(44.5)	0.241	1.16(0.91–1.49)	42(51.9)	5(45.5)	0.971	1.03(0.23–4.59)
AA	117(18.5)	112(18.5)	0.633	1.08(0.78–1.49)	10(12.3)	2(18.2)	0.327	0.32(0.03–3.13)
Allele								
G	730(57.8)	717(59.3)			100(61.7)	13(59.1)		
A	534(42.2)	493(40.7)	0.475	1.06(0.90–1.24)	62(38.3)	9(40.9)	0.686	0.82(0.32–2.10)
Genetic model								
Dominant			0.283	1.14(0.90–1.44)			0.969	0.97(0.24–3.91)
Recessive			0.967	0.75(0.75–1.33)			0.426	0.49(0.09–2.81)

SNPs, single nucleotide polymorphisms; CI, confidence interval; OR, odds ratio; IGRA, interferon gamma release assay.
+/-positive/negative.
$^{\#}$ adjusted by age, smoking and sex status.
* in tuberculosis group.

Table 4
Haplotype analyses in this study.

Gene/haplotype	Case(%) n = 1264	Control(%) n = 1210	*P*	OR(95%CI)
IFNG				
AC	450.0(35.6)	373.9(30.9)	0.013	1.24 (1.05–1.46)
AT	640.1(50.6)	660.1(54.6)	0.051	0.86 (0.73–1.00)
GT				
Other*	0.05(0)	0.09(0)		
IFNGR1				
CTGG	304.6(24.1)	317.6(26.2)	0.310	0.91 (0.76–1.09)
TCAG	152.5(12.1)	139.0(11.5)	0.557	1.08 (0.84–1.38)
TCGA	514.4(40.7)	484.9(40.1)	0.528	1.05 (0.90–1.24)
TTGG	250.6(19.8)	246.4(20.4)	0.886	0.99 (0.81–1.20)
Other*	41.9(3.4)	22.2(1.8)		

CI, confidence interval; OR, odds ratio.
* Those lowest frequency thresholds (LFT) < 0.03 were pooled in this part.

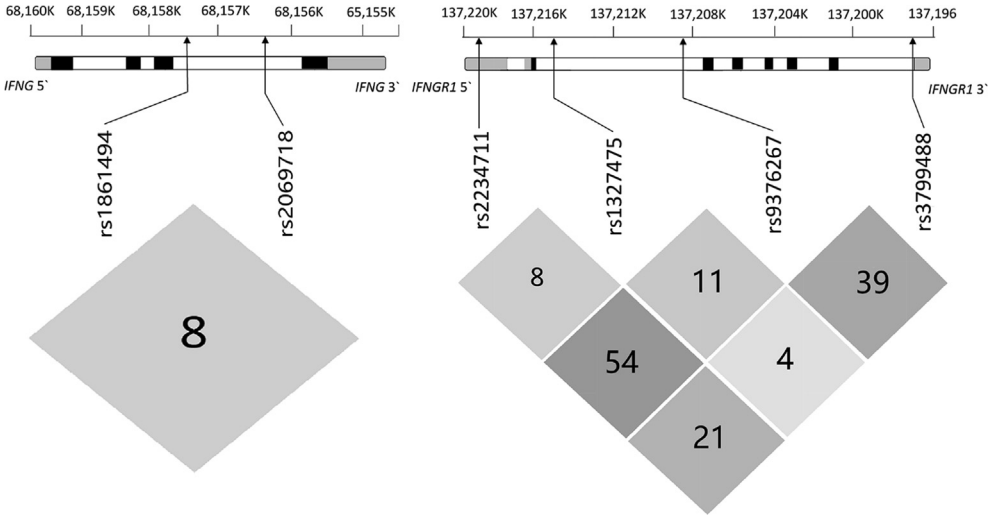

Fig. 1. 1. Map of the SNPs on the two chromosomes. The figure shows that *IFNG* (left) is located at 681,547,70 – 681,597,41 of chromosome 12 and *IFNGR1* (right) is located at 137,197,484 – 137,220,351 of chromosome 6. The black and gray boxes represent the exons, and white represent introns. Locations of the SNPs and the start and stop codons are indicated by arrows. Below, linkage disequilibrium (LD) of *IFNG* and *IFNGR1* gene polymorphisms. The r^2 values for all pairs single-nucleotide polymorphisms (SNPs) are shown as the percentage. When pairs SNPs are a complete link, the r^2 value is 1.

Compared with TT genotype, CT (OR = 1.28, 95%CI: 1.01–1.63; P = 0.040) and CC (OR = 1.51, 95%CI: 1.04–2.19; P = 0.031) were also risk factors for TB. Besides, rs1861494 was found to be associated with TB under a dominant model (OR = 1.33, 95%CI: 1.06–1.66; P = 0.013). None of the polymorphisms within *IFNGR1* achieved a significant difference in the allele and genotype distributions between TB cases and healthy controls (Table 3).

Haplotypes analyses revealed that *IFNG* AC haplotype exhibited significant correlation with TB susceptibility (Table 4). LD mapping indicates no high LD exists between all SNPs (r^2 < 0.8) (Fig. 1).

3.3. Analyses of polymorphisms and anti-TB treatment outcome

All TB patients were followed-up to obtain the treatment outcomes. IFNG rs1861494 C allele contributed to a 30% decrease in the falling treatment outcome (OR = 0.70, 95%CI: 0.52–0.94; P = 0.017) (Table 5). We also found CT genotype (OR = 0.64, 95%CI: 0.42–0.96; P = 0.030) and dominant model (OR = 0.62, 95%CI: 0.42–0.91; P = 0.014) were associated with treatment results.

3.4. Stratification analyses of polymorphisms and risk of TB

To assess the association between polymorphisms in the two genes and TB according to different ages and sexes, we conducted the subgroup analyses (Table 6). We observed rs1861494 allele C was a risk factor for TB among male (OR = 1.31, 95%CI: 1.03–1.66; P = 0.030) and younger subgroups (OR = 2.40, 95%CI: 1.70–3.38; P < 0.001).

3.5. Association between polymorphisms and QFT-GIT results

In the 92 active TB patients, we detected 88.0% of them had a positive QFT-GIT result. We then assessed the association between the six SNPs and QFT-GIT test results. No significant trend of QFT-GIT-positive associated with *IFNG* and *IFNGR1* polymorphisms (Table 3). We also analysed the influence of *IFNG* and *IFNGR1* genotypes on the IFNG production in QFT-GIT. Only rs2234711 genotypes were found to be associated with IFNG production levels. Compared with rs2234711 AA genotype, the GA (P = 0.001) and GG (p = 0.002) had higher IFNG production levels (Fig. 2).

4. Discussion

In the present study, we evaluated the association between polymorphisms in *IFNG* and its receptor *IFNGR1* and risk of TB in the Chinese population. Multiple logistic analysis suggested that IFNG rs1861494 allele and genotype were associated with an increased risk of TB, but there were no significant associations between SNPs within *IFNGR1* and TB.

When *M. TB* invades a host, IFNG activates macrophages and phagocytose pathogens. IFNG located on chromosome 12q15 exists four exons and three introns. *M. TB* infected mice with destroyed *IFNG* hardly induced the reactive nitrogen intermediates which could reduce the growth of the bacilli [5]). It was suggested that SNPs influence the IFNG expression via regulating the ability of binding with transcription factors [25–26]. Previous studies have researched the association between rs1861494 and rs2069718 and TB, with inconsistent results. He et al. suggested that these two loci were not significantly associated

Table 5
Association between genotype of *IFNG* and *IFNGR1* and treatment results.

Gene/SNPs	Failure, (%), n = 136	Success, (%), n = 496	$P^{\#}$	OR$^{\#}$(95%CI)
IFNG				
rs2069718A > G				
Genotype				
AA	97(71.3)	370(74.6)		
GA	38(27.9)	118(23.8)	0.299	1.26(0.82–1.94)
GG	1(0.7)	8(1.6)	0.435	0.43(0.05–3.55)
Allele				
A	232(85.3)	858(86.5)		
G	40(14.7)	134(13.5)	0.592	1.11(0.76–1.63)
Genetic model				
Dominant			0.402	1.20(0.78–1.84)
Recessive			0.396	0.40(0.05–3.29)
rs1861494T > C				
Genotype				
TT	69(50.7)	193(39.0)		
CT	53(39.0)	235(47.5)	0.030	0.64(0.42–0.96)
CC	14(10.3)	67(13.5)	0.067	0.54(0.28–1.04)
Allele				
T	191(70.2)	621(62.7)		
C	81(29.8)	369(37.3)	0.017	0.70(0.52–0.94)
Genetic model				
Dominant			0.014	0.62(0.42–0.91)
Recessive			0.226	0.68(0.37–1.27)
IFNGR1				
rs3799488T > C				
Genotype				
TT	77(56.6)	277(55.8)		
CT	51(37.5)	190(38.3)	0.981	0.99(0.67–1.49)
CC	8(5.9)	29(5.8)	0.923	1.04(0.46–2.38)
Allele				
T	205(75.4)	744(75.0)		
C	67(24.6)	248(25.0)	0.976	1.01(0.74–1.37)
Genetic model				
Dominant			0.986	0.99(0.68–1.47)
Recessive			0.909	1.05(0.47–2.36)
rs9376267C > T				
Genotype				
CC	45(33.1)	137(27.6)		
CT	62(45.6)	267(53.8)	0.139	0.72(0.46–1.11)
TT	29(21.3)	92(18.5)	0.981	0.99(0.58–1.71)
Allele				
C	152(55.9)	541(54.5)		
T	120(44.1)	451(45.5)	0.768	0.96(0.73–1.26)
Genetic model				
Dominant			0.249	0.79(0.52–1.18)
Recessive			0.421	1.21(0.76–1.95)
rs1327475G > A				
Genotype				
GG	106(77.9)	380(76.6)		
GA	26(19.1)	109(22.0)	0.560	0.87(0.54–1.40)
AA	4(2.9)	7(1.4)	0.279	2.01(0.57–7.07)
Allele				
G	238(87.5)	869(87.6)		
A	34(12.5)	123(12.4)	0.950	1.01(0.67–1.52)
Genetic model				
Dominant			0.773	0.94(0.59–1.48)
Recessive			0.265	2.04(0.58–7.10)
rs2234711G > A				
Genotype				
GG	46(33.8)	169(34.1)		
GA	60(44.1)	240(48.4)	0.651	0.91(0.59–1.40)
AA	30(22.1)	87(17.5)	0.553	1.18(0.69–2.01)
Allele				
G				
A			0.619	1.07(0.82–1.41)
Genetic model				
Dominant	152(55.9)	578(58.3)	0.937	0.98(0.66–1.47)
Recessive	120(44.4)	414(41.7)	0.326	1.27(0.79–2.03)

SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio.
$^{\#}$ adjusted by age, smoking and sex status.

Table 6
Subgroup analysis of *IFNG* and *IFNGR1* polymorphisms and TB.

Gene/SNPs	Genetic model	$P^{\#}$	OR$^{\#}$(95%CI)
IFNG			
rs2069718A > G	allele		
Male		0.392	0.87(0.63–1.20)
Female		0.976	1.01(0.73–1.39)
< 25		0.976	1.01(0.73–1.39)
≥25		0.887	0.98(0.75–1.28)
rs1861494T > C	allele		
Male		0.030	1.31(1.03–1.66)
Female		0.100	1.22(0.96–1.55)
< 25		< 0.001	2.40(1.70–3.38)
≥25		0.865	1.02(0.84–1.24)
IFNGR1			
rs3799488T > C	allele		
Male		0.493	0.91(0.71–1.18)
Female		0.555	0.93(0.72–1.19)
< 25		0.497	088(0.61–1.27)
≥25		0.588	0.94(0.77–1.16)
rs9376267C > T	allele		
Male		0.836	0.98(0.78–1.22)
Female		0.270	0.88(0.70–1.10)
< 25		0.119	0.78(0.57–1.07)
≥25		0.939	0.99(0.82–1.20)
rs1327475G > A	allele		
Male		0.797	0.96(0.68–1.34)
Female		0.274	1.22(0.86–1.74)
< 25		0.394	1.24(0.75–2.05)
≥25		0.822	1.03(0.78–1.37)
rs2234711G > A	allele		
Male		0.351	1.11(0.89–1.40)
Female		0.926	1.01(0.80–1.27)
< 25		0.276	1.19(0.87–1.62)
≥25		0.960	1.01(0.83–1.21)

SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio.
$^{\#}$ adjusted by age, smoking and sex status.

with TB [27]. A similar study made in Han Taiwanese reported that rs1861494 confer a risk of TB [28]. This locus has been demonstrated to be a functional SNP that could modulate DNA methylation and transcription factor complex formation [29]. Accorded with Lee et al., our results revealed that the C carrier of rs1861494 confers the risk of TB susceptibility [28]. However, our results were in disagreement with some other researches [10,30]. Our results were also different from a previous Chinese GWAS study which revealed three suggestive associated loci with TB [2]. The reasons of the discrepancy could be (1) our study was conducted in the southwest of China populations while subjects from the previous study were mainly from northern China; (2) SNPs that may have significance but not reach the genome-wide significant level in the previous study [31].

IFNGR1 was also widely researched regarding its association with TB. Animal and vitro studies demonstrated that *IFNGR1* plays a pivotal role in the progression of TB [32–33]. Individuals with *IFNGR1* gene defect were likely to be infected with *M. TB* disease [34]. Another study also reported humans with a Mendelian deficiency in *IFNGR1* are more susceptible to atypical *M.TB* infection [35]. In the present study, four tag SNPs were genotyped for analysis. A similar study has been conducted in a Chinese population, which detected a significant association between rs1327475 and rs2234711 and TB susceptibility. rs1327475 was also reported to be associated with systemic lupus erythematosus and schistosomal hepatic fibrosis [36–37]. rs2234711, located in the 5′-UTR of *IFNGR1*, encodes IFNGR ligand-binding chain I. This locus also acts as a binding site of transcription factors such as E2F6, ETS1, TBP, TAF1 and SIN3A, etc. [30]. However, in this study, none of the four tag SNPs within *IFNGR1* were associated with the risk of TB. Further studies with large sample size are needed to validate our study.

We evaluated whether *IFNG* and *IFNGR1* polymorphisms could

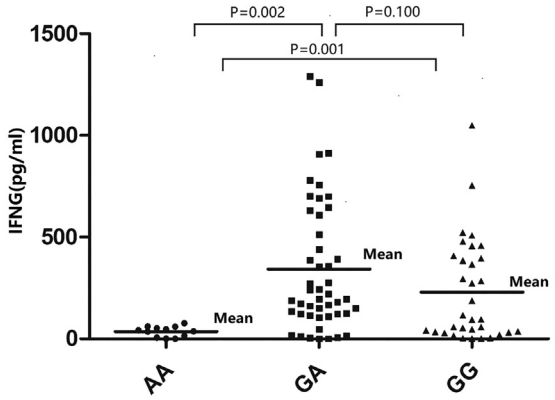

Fig. 2. The influence of *IFNGR1* rs2234711 genotypes on the IFNG production.

affect the anti-TB treatment outcome. We found that *IFNG* rs1861494 had significance in anti-TB treatment results. Compared with falling treatment outcome group, CT genotype, C allele and dominant model were protective factors for the successful treatment group. Besides, we conducted subgroups analysis based on gender and age. Interestingly, the *IFNG* rs1861494 polymorphism appears to be more noticeable in TB patients < 25 years. This finding was in lines with a published study of TB [38], in particular some other studies suggested that polymorphisms in *STAT4* and *TOX* affected the risk of pulmonary TB in subjects < 25 years [39–40]. We also detected rs1861494 was related to TB in male subgroup group, indicating that male is possible to be a phenotypic factor for those who more easily enter into active TB from *M. TB* infected status. Thus, more studies regarding the influence of gender and sex on TB are still needed.

Subsequently, we further revealed the association between *IFNG* and *IFNGR1* and QFT-GIT results. A total of 92 active TB in our study were measured QFT-GIT and the sensitivity was 81/92, or 88%. The result was similar to published data which reported the sensitivity of QFT-GIT in the diagnosis of active TB ranges from 60% to 89% [41–45]. Dai et al. pooled ten published studies assessing T-SPOT.TB in China and suggested the combined sensitivity was 88% [46] which was the same as our result. We demonstrated that there was no significant association between *IFNG* and *IFNGR1* polymorphisms and QFT-GIT results in this study. Our results were in agreement with those reported by a published study, which indicated that *IFNG* did not influence QFT-GIT results [47]. We then analysed the influence of genotype on IFNG production, suggesting that rs2234711 GA and GG genotypes had the higher IFNG production than that in AA genotype.

Nevertheless, several limitations should be addressed in this study. Firstly, our results suggested that only rs1861494 was associated with TB; however, rs1861494 was not associated with IFNG production levels. Secondly, our study lacks the functional validation of the rs1861494 which may alter levels of *IFNG* mRNA in the affected groups compared to healthy controls. And finally, the results were not validated in an independent cohort, which may result in the chance of type I errors.

In conclusion, we detected that *IFNG* rs1861494 polymorphism did influence the susceptibility to TB, particularly in the younger and male subgroups. In addition, *IFNGR1* rs2234711 genotypes could influence the IFNG production. Further studies with enlarging sample size are needed to validate the role of *IFNG* and *IFNGR1* polymorphisms in the risk of TB.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [grant number 81870015], and the National Scientific and Technological Major Project of China [grant number 2018ZX10715-003].

Declarations of Competing Interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.154775.

References

[1] World Health Organization, 2018. Global tuberculosis report 2018.
[2] H. Qi, Y.B. Zhang, L. Sun, C. Chen, B. Xu, F. Xu, et al., Discovery of susceptibility loci associated with tuberculosis in Han Chinese, Hum. Mol. Genet. 26 (23) (2017) 4752–4763.
[3] F.J. Kallmann, D. Reisner, Twin studies on the significance of genetic factors in tuberculosis, American Rev. Tubercul. Pulmonary Dis. 47 (6) (1943) 549–571.
[4] H.L. Collins, S.H. Kaufmann, The many faces of host responses to tuberculosis, Immunology 103 (1) (2001) 1–9.
[5] A.M. Cooper, D.K. Dalton, T.A. Stewart, J.P. Griffin, D.G. Russell, I.M. Orme, Disseminated tuberculosis in interferon gamma gene-disrupted mice, J. Exp. Med. 178 (6) (1993) 2243–2247.
[6] S. Dupuis, R. Doffinger, C. Picard, C. Fieschi, F. Altare, E. Jouanguy, et al., Human interferon-gamma-mediated immunity is a genetically controlled continuous trait that determines the outcome of mycobacterial invasion, Immunol. Rev. 178 (2000) 129–137.
[7] J. Lee, H. Kornfeld, Interferon-gamma Regulates the Death of M. tuberculosis-Infected Macrophages, J. Cell Death 3 (2010) 1–11.
[8] M. Cembrzynska-Nowak, E. Szklarz, A.D. Inglot, J.A. Teodorczyk-Injeyan, Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma, Am. Rev. Respir. Dis. 147 (2) (1993) 291–295.
[9] D.J. Dries, G.J. Jurkovich, R.V. Maier, T.P. Clemmer, S.N. Struve, J.A. Weigelt, et al., Effect of interferon gamma on infection-related death in patients with severe injuries. A randomized, double-blind, placebo-controlled trial, Arch. Surg. 129 (10) (1994) 1031–1041 discussion 42.
[10] A. Rolandelli, J.M. Pellegrini, N.O. Amiano, M.C. Santilli, M.P. Morelli, F.A. Castello, et al., The IFNG rs1861494 single nucleotide polymorphism is associated with protection against tuberculosis disease in argentina, Genes (Basel) 9 (1) (2018).
[11] Z. Araujo, A. Palacios, R. Biomon, B. Rivas-Santiago, C.J. Serrano, L. Enciso-Moreno, et al., Concordance between IFNgamma gene +874 A/T polymorphism and interferon-gamma expression in a TB-endemic indigenous setting, Rev. Soc. Bras. Med. Trop. 50 (2) (2017) 199–207.
[12] Z. Wei, S. Wenhao, M. Yuanyuan, L. Yang, Z. Daming, X. Jiangchun, et al., A single nucleotide polymorphism in the interferon-gamma gene (IFNG +874 T/A) is associated with susceptibility to tuberculosis, Oncotarget.. 8 (31) (2017) 50415–50429.
[13] A.G. Pacheco, C.C. Cardoso, M.O. Moraes, IFNG +874T/A, IL10 -1082G/A and TNF -308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study, Hum. Genet. 123 (5) (2008) 477–484.
[14] A.C. Greenlund, R.D. Schreiber, D.V. Goeddel, D. Pennica, Interferon-gamma induces receptor dimerization in solution and on cells, J. Biol. Chem. 268 (24) (1993) 18103–18110.
[15] O. Koch, A. Awomoyi, S. Usen, M. Jallow, A. Richardson, J. Hull, et al., IFNGR1 gene promoter polymorphisms and susceptibility to cerebral malaria, J. Infect. Dis. 185 (11) (2002) 1684–1687.
[16] H.S. Mohamed, M.E. Ibrahim, E.N. Miller, C.S. Peacock, E.A. Khalil, H.J. Cordell, et al., Genetic susceptibility to visceral leishmaniasis in The Sudan: linkage and association with IL4 and IFNGR1, Genes Immun. 4 (5) (2003) 351–355.
[17] P. Canedo, G. Corso, F. Pereira, N. Lunet, G. Suriano, C. Figueiredo, et al., The interferon gamma receptor 1 (IFNGR1) -56C/T gene polymorphism is associated with increased risk of early gastric carcinoma, Gut. 57 (11) (2008) 1504–1508.
[18] T. Thye, G.D. Burchard, M. Nilius, B. Muller-Myhsok, R.D. Horstmann, Genomewide linkage analysis identifies polymorphism in the human interferon-gamma receptor affecting Helicobacter pylori infection, Am. J. Hum. Genet. 72 (2) (2003) 448–453.
[19] E. Catherinot, C. Fieschi, J. Feinberg, J.L. Casanova, L.J. Couderc, Genetic susceptibility to mycobacterial disease: Mendelian disorders of the interleukin-12 -interferon-gamma axis, Rev. Mal. Respir. 22 (5 Pt 1) (2005) 767–776.
[20] P. Farnia, J. Ghanavi, P. Tabasri, S. Saif, A.A. Velayati, The importance of single nucleotide polymorphisms in interferon gamma receptor-1 gene in pulmonary patients infected with rapid grower mycobacterium, Int. J. Mycobacteriol. 5 (2016) S210–S211.
[21] G.S. Cooke, S.J. Campbell, J. Sillah, P. Gustafson, B. Bah, G. Sirugo, et al., Polymorphism within the interferon-gamma/receptor complex is associated with pulmonary tuberculosis, Am J Respir Crit Care Med. 174 (3) (2006) 339–343.
[22] J.G. Shin, B.L. Park, L.H. Kim, S. Namgoong, J.O. Kim, H.S. Chang, et al., Association study of polymorphisms in interferon-gamma receptor genes with the risk of pulmonary tuberculosis, Mol. Med. Rep. 12 (1) (2015) 1568–1578.
[23] M. Shamsi, M.R. Zolfaghari, P. Farnia, Association of IFN-gamma and P2X7 receptor

gene polymorphisms in susceptibility to tuberculosis among iranian patients, Acta Microbiol. Immunol. Hung. 63 (1) (2016) 93–101.
[24] J. Veen, M. Raviglione, H.L. Rieder, G.B. Migliori, P. Graf, M. Grzemska, et al., Standardized tuberculosis treatment outcome monitoring in Europe. Recommendations of a Working Group of the World Health Organization (WHO) and the European Region of the International Union Against Tuberculosis and Lung Disease (IUATLD) for uniform reporting by cohort analysis of treatment outcome in tuberculosis patients, Eur Respir J. 12 (2) (1998) 505–510.
[25] M. Rossouw, H.J. Nel, G.S. Cooke, P.D. van Helden, E.G. Hoal, Association between tuberculosis and a polymorphic NFkappaB binding site in the interferon gamma gene, Lancet 361 (9372) (2003) 1871–1872.
[26] J.H. Bream, A. Ping, X. Zhang, C. Winkler, H.A. Young, A single nucleotide polymorphism in the proximal IFN-gamma promoter alters control of gene transcription, Genes Immun. 3 (3) (2002) 165–169.
[27] J. He, J. Wang, D. Lei, S. Ding, Analysis of functional SNP in ifng/ifngr1 in Chinese Han population with tuberculosis, Scand. J. Immunol. 71 (6) (2010) 452–458.
[28] S.W. Lee, T.Y. Chuang, H.H. Huang, K.F. Lee, T.T. Chen, Y.H. Kao, et al., Interferon gamma polymorphisms associated with susceptibility to tuberculosis in a Han Taiwanese population, J. Microbiol. Immunol. Infect. 48 (4) (2015) 376–380.
[29] R. Gonsky, R.L. Deem, C.J. Landers, T. Haritunians, S. Yang, S.R. Targan, IFNG rs1861494 polymorphism is associated with IBD disease severity and functional changes in both IFNG methylation and protein secretion, Inflamm. Bowel. Dis. 20 (10) (2014) 1794–1801.
[30] J. Lu, H. Pan, Y. Chen, S. Tang, Y. Feng, S. Qiu, et al., Genetic polymorphisms of IFNG and IFNGR1 in association with the risk of pulmonary tuberculosis, Gene 543 (1) (2014) 140–144.
[31] I. Haq, S. Chappell, S.R. Johnson, J. Lotya, L. Daly, K. Morgan, et al., Association of MMP-2 polymorphisms with severe and very severe COPD: a case control study of MMPs-1, 9 and 12 in a European population, BMC Med. Genet. 11 (2010) 7.
[32] E. Jouanguy, S. Lamhamedi-Cherradi, F. Altare, M.C. Fondaneche, D. Tuerlinckx, S. Blanche, et al., Partial interferon-gamma receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guerin infection and a sibling with clinical tuberculosis, J. Clin. Invest. 100 (11) (1997) 2658–2664.
[33] F. Altare, E. Jouanguy, S. Lamhamedi-Cherradi, M.C. Fondaneche, C. Fizame, F. Ribierre, et al., A causative relationship between mutant IFNgR1 alleles and impaired cellular response to IFNgamma in a compound heterozygous child, Am. J. Hum. Genet. 62 (3) (1998) 723–726.
[34] M.J. Newport, C.M. Huxley, S. Huston, C.M. Hawrylowicz, B.A. Oostra, R. Williamson, et al., A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection, N. Engl. J. Med. 335 (26) (1996) 1941–1949.
[35] J.L. Casanova, L. Abel, The human model: a genetic dissection of immunity to infection in natural conditions, Nat. Rev. Immunol. 4 (1) (2004) 55–66.
[36] G.B. Nordang, M.K. Viken, S.S. Amundsen, E.S. Sanchez, B. Flato, O.T. Forre, et al., Interferon regulatory factor 5 gene polymorphism confers risk to several rheumatic diseases and correlates with expression of alternative thymic transcripts, Rheumatology (Oxford) 51 (4) (2012) 619–626.
[37] R.E. Blanton, E.A. Salam, A. Ehsan, C.H. King, K.A. Goddard, Schistosomal hepatic fibrosis and the interferon gamma receptor: a linkage analysis using single-nucleotide polymorphic markers, Eur. J. Hum. Genet. 13 (5) (2005) 660–668.
[38] A. Alcais, L. Quintana-Murci, D.S. Thaler, E. Schurr, L. Abel, J.L. Casanova, Life-threatening infectious diseases of childhood: single-gene inborn errors of immunity? Ann. N Y Acad. Sci. 1214 (2010) 18–33.
[39] A. Sabri, A.V. Grant, K. Cosker, S. El Azbaoui, A. Abid, I. Abderrahmani Rhorfi, et al., Association study of genes controlling IL-12-dependent IFN-gamma immunity: STAT4 alleles increase risk of pulmonary tuberculosis in Morocco, J. Infect. Dis. 210 (4) (2014) 611–618.
[40] A.V. Grant, J. El Baghdadi, A. Sabri, S. El Azbaoui, K. Alaoui-Tahiri, I. Abderrahmani Rhorfi, et al., Age-dependent association between pulmonary tuberculosis and common TOX variants in the 8q12-13 linkage region, Am. J Hum. Genet. 92 (3) (2013) 407–414.
[41] S. Zhang, L. Shao, L. Mo, J. Chen, F. Wang, C. Meng, et al., Evaluation of gamma interferon release assays using Mycobacterium tuberculosis antigens for diagnosis of latent and active tuberculosis in Mycobacterium bovis BCG-vaccinated populations, Clin. Vaccine Immunol. 17 (12) (2010) 1985–1990.
[42] H. Xia, X. Wang, F. Li, C. Longuet, G. Vernet, D. Goletti, et al., Diagnostic values of the QuantiFERON-TB Gold In-tube assay carried out in China for diagnosing pulmonary tuberculosis, PloS one. 10 (4) (2015) e0121021.
[43] M. Pai, A. Zwerling, D. Menzies, Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update, Ann. Intern. Med. 149 (3) (2008) 177–184.
[44] M. Sester, G. Sotgiu, C. Lange, C. Giehl, E. Girardi, G.B. Migliori, et al., Interferon-gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis, Eur. Respir. J. 37 (1) (2011) 100–111.
[45] Y. Kobashi, K. Mouri, S. Yagi, Y. Obase, M. Fukuda, N. Miyashita, et al., Usefulness of the QuantiFERON TB-2G test for the differential diagnosis of pulmonary tuberculosis, Intern. Med. 47 (4) (2008) 237–243.
[46] Y. Dai, Y. Feng, R. Xu, W. Xu, W. Lu, J. Wang, Evaluation of interferon-gamma release assays for the diagnosis of tuberculosis: an updated meta-analysis, European J. Clin. Microbiol. Infect. Dis. Off. Publicat. European Soc. Clin. Microbiol. 31 (11) (2012) 3127–3137.
[47] S. Kiazyk, L. Larcombe, C. Lopez, S. Matyas, C. Mesa, P. Orr, et al., IFN-gamma promoter polymorphisms do not affect QuantiFERON(R) TB Gold In-Tube test results in a Canadian population, Int. J. Tubercul. Dis. Off. J. Int. Union Tubercul. Lung Dis. 20 (12) (2016) 1647–1652.