

The Methylation in *B7-H4* and *BTLA* Genes are Associated with the Risk of Pulmonary Tuberculosis

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Background: The important roles of B7 homologous body 4 (*B7-H4*), B and T lymphocyte attenuator (*BTLA*) in patients with pulmonary tuberculosis (PTB) have been reported. This study aims to evaluate the association among *B7-H4* and *BTLA* genes polymorphism, methylation and PTB susceptibility.

Methodology: Here, we assessed the possible relationship of 10 single nucleotide polymorphisms (SNPs) in *B7-H4*, *BTLA* genes with PTB susceptibility in a Chinese population (496 PTB patients and 502 controls) by SNPscan technique. Then, the *B7-H4*, *BTLA* genes methylation levels among 98 PTB patients and 97 controls were detected using MethylTarget technique.

Results: This study found no significant differences in allele and genotype frequencies of *B7-H4* gene rs10754339, rs10801935, rs10923223, rs1937956, rs3738414, *BTLA* gene rs1982809, rs2971205, rs75368388, rs9288953 variants between PTB patients and controls. Haplotype analysis suggested that the lower frequencies of *B7-H4* AATTG haplotype, *BTLA* GATT haplotype and the higher frequency of *BTLA* AGTC haplotype were found in PTB patients when compared with controls. We also found that the frequency of *BTLA* gene rs9288953 C allele was significantly increased in PTB patients with drug resistance. Moreover, the methylation levels of *B7-H4* and *BTLA* genes in PTB patients were greater than that in controls, and rs10754339 variant in *B7-H4* gene could affect its methylation level in PTB patients.

Conclusion: *B7-H4*, *BTLA* genes polymorphism might not affect PTB susceptibility, while the abnormal methylation levels of *B7-H4*, *BTLA* genes were associated with the genetic background of PTB.

Keywords: pulmonary tuberculosis, single nucleotide polymorphisms, DNA methylation, *B7-H4*, *BTLA*

Introduction

Tuberculosis (TB) is a common, serious infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) infection, which is still a major public health problem. Before the COVID-19 pandemic, TB was the leading cause of death from a single pathogen, and the most common type of this disease was pulmonary TB (PTB).¹ The COVID-19 pandemic resulted in reduced access to TB diagnosis and treatment. There were approximately 10.6 million new cases of TB worldwide and about 0.78 million new cases of TB in China in 2021 according to the Global Tuberculosis Report 2022.² Studies showed that about a quarter of the population was infected with *M. tuberculosis* globally, while less than 10% of those infected would develop active TB.³ This suggested that the pathogenesis of TB was influenced by multiple factors, including nutrition, immunity, genetic factors, smoke, alcohol, and HIV infection.⁴⁻⁶

Genome-wide association studies and twin studies had confirmed the important influence of host genetics on TB susceptibility.^{7,8} In addition, candidate gene association studies had found many important variants related to TB susceptibility, most of which were mainly located in immune-related genes, such as Toll-like receptor genes, vitamin D metabolic pathway genes and human leukocyte antigen genes.⁹⁻¹¹ However, these studies only could explain part of the heritability of TB. Epigenetics also showed the important roles in TB development. For instance, abnormal DNA methylation, which was known as an important epigenetic mode, of several genes was considered to affect the development of TB.¹²

The PTB development was affected by the host's immune function, and host control of *M. tuberculosis* infection required innate and adaptive immune responses. B- and T-lymphocyte attenuator (*BTLA*) and B7 homologous body 4 (*B7-H4*) were two newly discovered immune checkpoint inhibitors.^{13,14} *BTLA* was mainly expressed in dendritic cells (DC), T cells, B cells and some myeloid cells. The interaction between *BTLA* and its ligand herpesvirus entry mediator (HVEM) could inhibit the proliferation and activation of T cells, as well as the expression of a variety of cytokines in T cells.¹⁵ The function of *BTLA* in PTB had been investigated, and *BTLA* exerted immune memory on $\alpha\beta$ T cells in active PTB.¹⁶ *B7-H4* was inductively expressed by immune cells such as T cells, B cells, DC cells and macrophages. The increased expression of *B7-H4* in DC might be associated with the increase of Tregs, and could influence the differentiation of naive T cells, thereby affecting the identification of *M. tuberculosis* by the immune system and inducing anti-TB specific immune responses.¹⁷ *BTLA* and *B7-H4* expression levels were abnormally increased in CD11c⁺ antigen presenting cells from peripheral blood among PTB patients, suggesting that these factors were closely involved in the pathogenesis of PTB.¹⁸

Previous studies revealed that genetic variation in *B7-H4*, *BTLA* was related the susceptibility to many diseases, including autoimmune thyroid diseases,¹⁹ ankylosing spondylitis (AS),²⁰ breast cancer.²¹ Nevertheless, the genetic effect of *B7-H4*, *BTLA* genes on the risk of PTB had not yet been explored. Therefore, we conducted this study to investigate the association among several single nucleotide polymorphisms (SNPs) in *B7-H4*, *BTLA* genes, as well as their methylation level, and PTB susceptibility in the Chinese Han population.

Materials and Methods

PTB Patients and Controls

We continuously collected the patients with PTB from Anhui Chest Hospital, and all patients were diagnosed by senior clinicians according to the diagnosis for PTB (WS 288–2017) and the classification of TB (WS 196–2017) from the Health Industry Standard of the People's Republic of China. Exclusion criteria of PTB patients were: HIV-positive, other infectious diseases, autoimmune diseases and cancer. In this study, we selected healthy volunteers from the local health examination center as control group, and all of whom had no history of infectious diseases, pulmonary diseases, bacterial or viral infections.

The study was conducted in compliance with the provisions of the Declaration of Helsinki and approved by the Ethics Committee of Anhui Chest Hospital (K2023-011). Written informed consent was obtained from all participants, peripheral blood (3–5 mL), and clinical data were collected. Genomic DNA was extracted from peripheral blood using the Flexi Gene-DNA Kit (Qiagen, Valencia, CA). The required clinical data included fever, drug resistance, drug-induced liver injury (DILI), pulmonary infection, leukopenia, sputum smear, etc.

SNP Selection and Genotyping

A total of ten SNPs, including *B7-H4* gene rs10754339, rs10801935, rs10923223, rs1937956, rs3738414 and *BTLA* gene rs9288952, rs1982809, rs2971205, rs75368388, rs9288953 were genotyped by SNPscan technique, with technical support from the Center for Genetic and Genomic Analysis, Genesky Biotechnologies Inc., Shanghai. Only participants with 100% genotyping success for the selected SNPs were included in the final analysis.

The selection of these SNPs was mainly based on the following principles of screening tag SNPs. The tag SNPs of *B7-H4* and *BTLA* genes was selected using genetic data from Ensembl genome browser 85 and CHBS_1000g with Haploview 4.0 software (Cambridge, MA, USA) based on following strategy: (1) minor allele frequency ≥ 0.05 ; (2) an r^2 cutoff of 0.8 for linkage disequilibrium; (3) the flanking 2.0 kb regions of each gene.

Methylation Detecting

In this study, DNA samples were first treated with bisulfite, and then used for targeted region methylation sequencing to the promoter regions in *B7-H4* and *BTLA* genes. The CpG islands of the target fragments in the promoter regions of these two genes were sequenced using the MethylTarget technique with the support of the Genesky Biotechnology (Shanghai) Center for Genetic and Genome Analysis. Only one specific target fragment was respectively detected on *B7-H4* and

BTLA genes, and the primer sequence of these fragments were shown in [Table S1](#). The methylation level of every specific target fragment could be got through calculating the mean methylation level of all CpG sites on the fragment.

Statistical Analysis

We used the chi-squared test to assess whether the genotype distribution of 10 SNPs in *B7-H4* and *BTLA* genes in the control group achieved Hardy–Weinberg Equilibrium (HWE). The distributions of allele and genotype between PTB patients and controls were compared by logistic regression model, and odds ratios (OR) with 95% confidence intervals (CI) was calculated. The association of *B7-H4* and *BTLA* genes variation with PTB susceptibility was analyzed under two genetic models (dominant model and recessive model), and haplotype analysis was conducted using SHEsis software.²² The methylation levels of *B7-H4* and *BTLA* genes were expressed by median (P_{25} , P_{75}). The differences of *B7-H4* and *BTLA* genes methylation levels between different groups were analyzed by Mann–Whitney *U*-test and Kruskal–Wallis *H*-test. All statistical analyses were calculated with SPSS 23.0, and a *P*-value <0.05 was regarded as statistically significant. Bonferroni correction was used for multiple testing in SNP analysis, and $P < 0.0055$ (0.05/9) was considered as statistically significant.

Results

Comparison of *B7-H4* and *BTLA* Genes Polymorphisms Among PTB Patients and Controls

Ten SNPs in *B7-H4* and *BTLA* genes was successfully genotyped in 496 patients with PTB and 502 controls. The differences regarding age (an average age of patients: 44.73 ± 18.04 years, an average age of controls: 44.42 ± 3.50 years) and gender (patients: 183 female/313 male; controls: 201 female/301 male) distribution between PTB patients and controls showed no significant. HWE test was performed for the genotype distribution of all SNPs in controls, and the results showed that nine SNPs exhibited HWE except *BTLA* gene rs9288952.

Our results revealed that GA genotype frequency of rs3738414 variant in *B7-H4* gene was increased in PTB patients when compared to controls ($P=0.030$ after adjustment of gender and age), while the difference was not statistically significant after Bonferroni correction ($P > 0.0055$) ([Table 1](#)). Similarly, there was no statistical association between the allele, genotype frequencies of other SNPs (rs10754339, rs1937956, rs10923223, rs10801935) in *B7-H4* gene and PTB susceptibility. For *BTLA* gene, the frequencies of AA genotype in rs1982809 variant, GG genotype in rs2971205 variant, C allele in rs9288953 variant seemed to be increased in PTB patients than that in controls ($P=0.039$, $P=0.018$, $P=0.045$ after adjustment of gender and age), but no statistically significant association was found after Bonferroni correction ($P > 0.0055$) ([Table 1](#)). The association among *B7-H4*, *BTLA* genes polymorphism and PTB susceptibility was also assessed under dominant model, recessive mode, and no association reached statistical significance after Bonferroni correction ($P > 0.0055$).

We constructed the haplotypes of *B7-H4* and *BTLA* genes by SHEsis software, and only the haplotypes with a frequency more than 3% were included in [Table 2](#). The results of haplotype analysis showed that when compared with controls, AATTG haplotype of *B7-H4* gene and GATT haplotype of *BTLA* gene had significantly decreased frequencies ($P=0.033$, $P=0.045$) in PTB patients, while AGTC haplotype of *BTLA* gene had an increased frequency ($P=0.002$). Other haplotypes of *B7-H4* and *BTLA* genes did not exhibit any significant association with PTB susceptibility.

Association Between *B7-H4*, *BTLA* Genes Polymorphisms and Several Clinical Manifestations in PTB Patients

We respectively divided PTB patients into two groups based on whether the patients had specific clinical features or not, such as fever, drug resistance, DILI, pulmonary infection, hypoproteinemia, sputum smear-positive. Then, we analyzed the association between the allele and genotype frequencies of all SNPs in *B7-H4*, *BTLA* genes and above clinical manifestations in PTB patients. The results demonstrated that the frequency of *BTLA* gene rs9288953 C allele was significantly increased in PTB patients with drug resistance in comparison with the patients without drug resistance ($P=0.035$) ([Table 3](#)). However, other SNPs in *B7-H4* and *BTLA* genes were not associated with clinical manifestations of PTB.

Table I Association of *B7-H4*, *BTLA* Genes Polymorphisms with PTB Susceptibility

SNP	Analyze Model		Controls	PTB patients	P-value	OR (95%CI)
<i>B7-H4</i>						
rs10754339	Genotype	GG	4 (0.80)	8 (1.61)	0.265	1.990 (0.593–6.670) ^a
		GA	84 (16.73)	77 (15.52)	0.648	0.924 (0.659– 1.296) ^a
		AA	414 (82.47)	411 (82.86)	Reference	
	Allele	G	92 (9.16)	93 (9.38)	0.870	1.028 (0.740– 1.427)
		A	912 (90.84)	899 (90.63)	Reference	
	Dominant model	AA	414 (82.47)	411 (82.86)	0.870	0.995 (0.940– 1.053) ^a
		GA+GG	88 (17.53)	85 (17.14)	Reference	
Recessive model	GG	4 (0.80)	8 (1.61)	0.253	2.024 (0.605– 6.772) ^a	
	GA+AA	498 (99.20)	488 (98.39)	Reference		
rs10801935	Genotype	CC	18 (3.59)	26 (5.24)	0.176	1.536 (0.825– 2.860) ^a
		CA	156 (31.08)	160 (32.26)	0.553	1.085 (0.828– 1.421) ^a
		AA	328 (65.34)	310 (62.50)	Reference	
	Allele	C	192 (19.12)	212 (21.37)	0.211	1.029 (0.984– 1.075)
		A	812 (80.88)	780 (78.63)	Reference	
	Dominant model	AA	328 (65.34)	310 (62.50)	0.324	0.879 (0.681– 1.136) ^a
		CA+CC	174 (34.66)	186 (37.50)	Reference	
Recessive model	CC	18 (3.59)	19 (3.83)	0.200	1.495 (0.809– 2.765) ^a	
	CA+AA	484 (96.41)	470 (94.76)	Reference		
rs10923223	Genotype	CC	87 (17.33)	97 (19.56)	0.541	1.121 (0.777– 1.619) ^a
		CT	262 (52.19)	248 (50.00)	0.723	0.950 (0.714– 1.263) ^a
		TT	153 (30.48)	151 (30.44)	Reference	
	Allele	C	436 (43.43)	442 (44.56)	0.611	1.020 (0.944– 1.103)
		T	568 (56.57)	550 (55.44)	Reference	
	Dominant model	TT	153 (30.48)	151 (30.44)	0.957	1.007 (0.769– 1.320) ^a
		Ct+CC	349 (69.52)	345 (69.56)	Reference	
Recessive model	CC	87 (17.33)	97 (19.56)	0.359	1.162 (0.843– 1.601) ^a	
	CT+TT	415 (82.67)	399 (80.44)	Reference		
rs1937956	Genotype	AA	7 (1.39)	9 (1.81)	0.632	1.277 (0.470– 3.471) ^a
		AT	100 (19.92)	99 (19.96)	0.957	1.009 (0.739– 1.377) ^a
		TT	395 (78.69)	388 (78.23)	Reference	
	Allele	A	114 (11.35)	117 (11.79)	0.759	1.005 (0.974– 1.037)
		T	890 (88.65)	875 (88.21)	Reference	
	Dominant model	TT	395 (78.69)	388 (78.23)	0.866	0.974 (0.720– 1.319) ^a
		AT+AA	107 (21.31)	108 (21.77)	Reference	
Recessive model	AA	7 (1.39)	9 (1.81)	0.611	1.295 (0.478– 3.510) ^a	
	AT+TT	495 (98.61)	487 (98.19)	Reference		
rs3738414	Genotype	AA	36 (7.17)	32 (6.45)	0.982	1.006 (0.607– 1.668) ^a
		GA	181 (36.06)	212 (42.74)	0.030	1.337 (1.029– 1.736) ^a
		GG	285 (56.77)	252 (50.81)	Reference	
	Allele	A	253 (25.20)	276 (27.82)	0.184	1.036 (0.983–1.092)
		G	751 (74.80)	716 (72.18)	Reference	
	Dominant model	GG	285 (56.77)	252 (50.81)	0.052	0.780 (0.608–1.002) ^a
		GA+AA	217 (43.23)	244 (49.19)	Reference	
Recessive model	AA	36 (7.17)	32 (6.45)	0.651	0.892 (0.545–1.461) ^a	
	GA+GG	466 (92.83)	464 (93.55)	Reference		

(Continued)

Table 1 (Continued).

SNP	Analyze Model	Controls	PTB patients	P-value	OR (95%CI)	
BTLA						
rs1982809	Genotype	AA	17 (3.39)	30 (6.05)	0.039	1.915 (1.032–3.554) ^a
		AG	176 (35.06)	180 (36.29)	0.442	1.109 (0.852–1.442) ^a
		GG	309 (61.55)	286 (57.66)	Reference	
	Allele	A	210 (20.92)	240 (24.19)	0.080	1.043 (0.995–1.094)
		G	794 (79.08)	752 (75.81)	Reference	
	Dominant model	GG	309 (61.55)	286 (57.66)	0.201	0.848 (0.658–1.092) ^a
		AG+AA	193 (38.45)	210 (42.34)	Reference	
	Recessive model	AA	17 (3.39)	30 (6.05)	0.054	1.827 (0.993–3.363) ^a
		AG+GG	485 (96.61)	466 (93.95)	Reference	
	rs2971205	Genotype	GG	13 (2.59)	28 (5.65)	0.018
AG			165 (32.87)	162 (32.66)	0.738	1.047 (0.801–1.369) ^a
AA			324 (64.54)	306 (61.69)	Reference	
Allele		G	191 (19.02)	218 (21.98)	0.102	1.038 (0.993–1.085)
		A	813 (80.98)	774 (78.02)	Reference	
Dominant model		AA	324 (64.54)	306 (61.69)	0.327	0.879 (0.679–1.138) ^a
		AG+GG	178 (35.46)	190 (38.31)	Reference	
Recessive model		GG	13 (2.59)	28 (5.65)	0.018	2.242 (1.146–4.383) ^a
		AG+AA	489 (97.41)	468 (94.35)	Reference	
rs75368388		Genotype	AA	0 (0)	2 (0.40)	0.999
	TA		69 (13.75)	65 (13.10)	0.780	0.949 (0.660–1.367) ^a
	TT		433 (86.25)	429 (86.49)	Reference	
	Allele	A	69 (6.87)	69 (6.96)	0.942	1.001 (0.977–1.025)
		T	935 (93.13)	923 (93.04)	Reference	
	Dominant model	TT	433 (86.25)	429 (86.49)	0.915	1.020 (0.710–1.464) ^a
		TA+AA	69 (13.75)	67 (13.51)	Reference	
	Recessive model	AA	0 (0)	2 (0.4)	0.999	— ^a
		TA+TT	502 (100)	494 (99.60)	Reference	
	rs9288953	Genotype	CC	100 (19.92)	113 (22.78)	0.054
CT			245 (48.80)	258 (52.02)	0.060	1.325 (0.989–1.777) ^a
TT			157 (31.27)	125 (25.20)	Reference	
Allele		C	445 (44.32)	484 (48.79)	0.045	1.087 (1.002–1.180)
		T	559(55.68)	508(51.21)	Reference	
Dominant model		TT	157 (31.27)	125 (25.20)	0.033	0.739 (0.560–0.975) ^a
		CT+CC	345 (68.73)	371 (74.80)	Reference	
Recessive model		CC	100 (19.92)	113 (22.78)	0.268	1.187 (0.876–1.608) ^a
		CT+TT	402 (80.08)	383 (77.22)	Reference	

Notes: ^aCalculated by nonconditional logistic regression model adjusted for gender and sex. P-value was corrected by Bonferroni correction (0.05/9), P<0.0055 was considered statistically significant.

Comparison of *B7-H4*, *BTLA* Genes Methylation Levels in PTB Patients and Controls

The methylation levels of *B7-H4*, *BTLA* genes were examined in 98 PTB patients and 97 controls. No difference in the distribution of age (an average age of patients: 43.52 ± 17.59 years, an average age of controls: 43.45 ± 4.49 years) or gender (patients: 45 female/53 male; controls: 46 female/51 male) between PTB patients and controls was found. In this study, only one specific target fragment was detected in *B7-H4* and *BTLA* genes, respectively. Therefore, the methylation level of every target fragment was used to represent the methylation level of *B7-H4* and *BTLA* genes in this study.

In PTB patients, the methylation levels of *B7-H4*, *BTLA* genes were 0.2966(0.2938–0.3003), 0.7735 (0.7171–0.8433). In the control group, the methylation level of *B7-H4*, *BTLA* genes were 0.2941(0.2914–0.7925), 0.7456 (0.7149–0.7925). As shown in Figures 1 and 2, we found that the methylation levels of *B7-H4*, *BTLA* genes in PTB patients were both

Table 2 Haplotype Analysis of *B7-H4*, *BTLA* Genes in PTB Patients

Haplotype	PTB Patients	Controls	P-value	OR (95%CI)
<i>B7-H4</i> rs10754339-rs10801935-rs10923223-rs1937956-rs3738414				
AACTG	274.13 (0.276)	265.10 (0.264)	0.331	1.105 (0.903~1.352)
AATTA	251.50 (0.254)	235.79 (0.235)	0.191	1.148 (0.933~1.414)
AATTG	226.08 (0.228)	276.01 (0.275)	0.033	0.799 (0.650~0.982)
ACCTG	86.07 (0.087)	104.04 (0.104)	0.267	0.843 (0.624~1.140)
GCCAG	53.06 (0.053)	43.22 (0.043)	0.224	1.291 (0.854~1.950)
<i>BTLA</i> rs10801935-rs10923223-rs1937956-rs3738414				
AATC	155.94 (0.157)	155.95 (0.155)	0.884	1.018 (0.799~1.298)
AGTC	51.31 (0.052)	25.54 (0.025)	0.002	2.099 (1.294~3.403)
GAAC	57.30 (0.058)	58.40 (0.058)	0.983	0.996 (0.684~1.450)
GATC	46.07 (0.046)	36.66 (0.037)	0.259	1.290 (0.828~2.010)
GATT	481.94 (0.486)	533.48 (0.531)	0.045	0.833 (0.696~0.996)
GGTC	161.70 (0.163)	158.90 (0.158)	0.748	1.040 (0.818~1.322)

Note: The haplotypes with a frequency <3% both in controls and PTB patients has been dropped.

higher than that in controls ($P=0.001$, $P=0.005$). We also analyzed the association among *B7-H4*, *BTLA* genes methylation levels and some clinical manifestations in PTB patients. This study did not obtain any significant association between *B7-H4*, *BTLA* genes methylation levels and the clinical manifestations, including fever, drug resistance, DILI, pulmonary infection, hypoproteinemia, sputum smear-positive (Table 4).

Association Between *B7-H4*, *BTLA* Genes Polymorphisms and Their Methylation Levels

This study conducted an association analysis to assess whether the genotype of each SNP of *B7-H4*, *BTLA* genes affected their methylation levels in 98 PTB patients (Table 5). Our results demonstrated that rs10754339 variant in *B7-H4* gene could affect its methylation level. In addition, no statistically significant association was found among *BTLA* gene polymorphism and its methylation level.

Discussion

As a complex infectious disease, genetic factors played an important role in the pathogenesis of PTB. Further elucidation of host genetic differences was an important way to understand the pathogenesis of PTB and formulate appropriate treatment, prognosis strategies. Recently, some studies had extensively studied the regulatory role of checkpoint inhibitory molecules *BTLA* and *B7-H4* in a variety of immune cells, and revealed their influence on the pathogenesis of PTB.^{16,23} The present study was the first to explore the potential association among *BTLA*, *B7-H4* genes variation and PTB susceptibility from the perspective of gene polymorphisms and epigenetics, and provided several previously undescribed findings.

B7-H4, also known as B7S1 and B7x, was the newest member of the B7 family, and could modulate antigen-specific immune responses by inhibiting T cell activation and the production of cytokines.²⁴ One previous research demonstrated that *B7-H4*-expressing macrophages was significantly increased in the peripheral blood from lung cancer patients when compared to TB patients and healthy controls.²⁵ In addition, the high expression of *B7-H4* was not only positively related to tumor progression, but also involved in tumor immune escape. Chen et al found that mature activated DCs expressed CD83 and several negative immune molecules, such as *B7-H4*, *BTLA*,²⁵ and an obvious proportion increase regarding the co-expression of *BTLA* and *B7-H4* was found in mDC from TB patients.¹⁷ Moreover, this study also implied that the high expression of *B7-H4* and *BTLA* might play a negative regulatory influence of the mDCs on anti-TB immunity through promoting the expression of CD83 and HLA-DR. Some studies were also conducted the potential connection and the polymorphism in *B7-H4* gene and susceptibility to human disease. Zhang et al performed an association study of rs10754339, rs10801935, rs3738414 variants in *B7-H4* gene in breast cancer, and found that these SNPs were connected

Table 3 Relationship Between *B7-H4*, *BTLA* Genes Polymorphisms and the Clinical Manifestations in PTB Patients

SNP	Allele	Clinical Features	Group	Genotype			Pvalue	Allele		P-value
	(Mm)			MM	Mm	mm		M	m	
<i>B7-H4</i>										
rs10754339	A/G	Fever	+	70 (84.34)	13 (15.66)	0 (0)	0.489	153 (92.17)	13 (7.83)	0.506
			-	341 (82.77)	64 (15.53)	7 (1.7)		746 (90.53)	78 (9.47)	
		Drug resistance	+	64 (86.49)	9 (12.16)	1 (1.35)	0.680	137 (92.57)	11 (7.43)	0.422
			-	347 (82.42)	68 (16.15)	6 (1.43)		762 (90.5)	80 (9.5)	
		DILI	+	55 (80.88)	12 (17.65)	1 (1.47)	0.875	122 (89.71)	14 (10.29)	0.632
			-	356 (83.37)	65 (15.22)	6 (1.41)		777 (90.98)	77 (9.02)	
		Pulmonary infection	+	50 (81.97)	11 (18.03)	0 (0)	0.531	111 (90.98)	11 (9.02)	0.943
	-	361 (83.18)	66 (15.21)	7 (1.61)		788 (90.78)	80 (9.22)			
		Hypoproteinemia	+	36 (87.8)	4 (9.76)	1 (2.44)	0.491	76 (92.68)	6 (7.32)	0.539
			-	375 (82.6)	73 (16.08)	6 (1.32)		823 (90.64)	85 (9.36)	
		Sputum smear-positive	+	113 (81.29)	24 (17.27)	2 (1.44)	0.879	250 (89.93)	28 (10.07)	0.703
			-	260 (83.07)	48 (15.34)	5 (1.6)		568 (90.73)	58 (9.27)	
rs10801935	A/C	Fever	+	54 (65.06)	26 (31.33)	3 (3.61)	0.766	134 (80.72)	32 (19.28)	0.504
			-	256 (62.14)	134 (32.52)	22 (5.34)		646 (78.4)	178 (21.6)	
		Drug resistance	+	51 (68.92)	19 (25.68)	4 (5.41)	0.415	121 (81.76)	27 (18.24)	0.338
			-	259 (61.52)	141 (33.49)	21 (4.99)		659 (78.27)	183 (21.73)	
		DILI	+	39 (57.35)	26 (38.24)	3 (4.41)	0.531	104 (76.47)	32 (23.53)	0.477
			-	271 (63.47)	134 (31.38)	22 (5.15)		676 (79.16)	178 (20.84)	
		Pulmonary infection	+	38 (62.3)	21 (34.43)	2 (3.28)	0.768	97 (79.51)	25 (20.49)	0.835
	-	272 (62.67)	139 (32.03)	23 (5.3)		683 (78.69)	185 (21.31)			
		Hypoproteinemia	+	28 (68.29)	10 (24.39)	3 (7.32)	0.460	66 (80.49)	16 (19.51)	0.694
			-	282 (62.11)	150 (33.04)	22 (4.85)		714 (78.63)	194 (21.37)	
		Sputum smear-positive	+	92 (66.19)	38 (27.34)	9 (6.47)	0.368	222 (79.86)	56 (20.14)	0.629
			-	193 (61.66)	105 (33.55)	15 (4.79)		491 (78.43)	135 (21.57)	
rs10923223	T/C	Fever	+	26 (31.33)	41 (49.4)	16 (19.28)	0.976	93 (56.02)	73 (43.98)	0.849
			-	124 (30.1)	207 (50.24)	81 (19.66)		455 (55.22)	369 (44.78)	
		Drug resistance	+	28 (37.84)	30 (40.54)	16 (21.62)	0.183	86 (58.11)	62 (41.89)	0.465
			-	122 (28.98)	218 (51.78)	81 (19.24)		462 (54.87)	380 (45.13)	
		DILI	+	23 (33.82)	30 (44.12)	15 (22.06)	0.568	76 (55.88)	60 (44.12)	0.894
			-	127 (29.74)	218 (51.05)	82 (19.2)		472 (55.27)	382 (44.73)	

(Continued)

Table 3 (Continued).

SNP	Allele	Clinical Features	Group	Genotype			Pvalue	Allele		P-value
	(Mm)			MM	Mm	mm		M	m	
rs1937956	T/A	Pulmonary infection	+	21 (34.43)	32 (52.46)	8 (13.11)	0.376	74 (60.66)	48 (39.34)	0.208
			-	129 (29.72)	216 (49.77)	89 (20.51)		474 (54.61)	394 (45.39)	
		Hypoproteinemia	+	18 (43.9)	17 (41.46)	6 (14.63)	0.138	53 (64.63)	29 (35.37)	0.078
			-	132 (29.07)	231 (50.88)	91 (20.04)		495 (54.52)	413 (45.48)	
		Sputum smear-positive	+	44 (31.65)	70 (50.36)	25 (17.99)	0.783	158 (56.83)	120 (43.17)	0.539
			-	89 (28.43)	164 (52.4)	60 (19.17)		342 (54.63)	284 (45.37)	
		Fever	+	66 (79.52)	17 (20.48)	0 (0)	0.441	149 (89.76)	17 (10.24)	0.544
			-	322 (78.16)	82 (19.9)	8 (1.94)		726 (88.11)	98 (11.89)	
		Drug resistance	+	60 (81.08)	13 (17.57)	1 (1.35)	0.829	133 (89.86)	15 (10.14)	0.542
			-	328 (77.91)	86 (20.43)	7 (1.66)		742 (88.12)	100 (11.88)	
rs3738414	G/A	DILI	+	53 (77.94)	14 (20.59)	1 (1.47)	0.987	120 (88.24)	16 (11.76)	0.954
			-	335 (78.45)	85 (19.91)	7 (1.64)		755 (88.41)	99 (11.59)	
		Pulmonary infection	+	48 (78.69)	13 (21.31)	0 (0)	0.553	109 (89.34)	13 (10.66)	0.724
			-	340 (78.34)	86 (19.82)	8 (1.84)		766 (88.25)	102 (11.75)	
		Hypoproteinemia	+	35 (85.37)	5 (12.2)	1 (2.44)	0.401	75 (91.46)	7 (8.54)	0.363
			-	353 (77.75)	94 (20.7)	7 (1.54)		800 (88.11)	108 (11.89)	
		Sputum smear-positive	+	107 (76.98)	30 (21.58)	2 (1.44)	0.889	244 (87.77)	34 (12.23)	0.915
			-	244 (77.96)	63 (20.13)	6 (1.92)		551 (88.02)	75 (11.98)	
		Fever	+	38 (45.78)	42 (50.6)	3 (3.61)	0.208	118 (71.08)	48 (28.92)	0.744
			-	213 (51.7)	170 (41.26)	29 (7.04)		596 (72.33)	228 (27.67)	
		Drug resistance	+	40 (54.05)	28 (37.84)	6 (8.11)	0.588	108 (72.97)	40 (27.03)	0.802
			-	211 (50.12)	184 (43.71)	26 (6.18)		606 (71.97)	236 (28.03)	
		DILI	+	37 (54.41)	25 (36.76)	6 (8.82)	0.456	99 (72.79)	37 (27.21)	0.851
			-	214 (50.12)	187 (43.79)	26 (6.09)		615 (72.01)	239 (27.99)	
		Pulmonary infection	+	30 (49.18)	25 (40.98)	6 (9.84)	0.519	85 (69.67)	37 (30.33)	0.519
			-	221 (50.92)	187 (43.09)	26 (5.99)		629 (72.47)	239 (27.53)	
		Hypoproteinemia	+	19 (46.34)	19 (46.34)	3 (7.32)	0.841	57 (69.51)	25 (30.49)	0.582
			-	232 (51.1)	193 (42.51)	29 (6.39)		657 (72.36)	251 (27.64)	
		Sputum smear-positive	+	68 (48.92)	62 (44.6)	9 (6.47)	0.910	198 (71.22)	80 (28.78)	0.687
			-	160 (51.12)	134 (42.81)	19 (6.07)		454 (72.52)	172 (27.48)	

BTLA										
rs1982809	G/A	Fever	+	53 (63.86)	24 (28.92)	6 (7.23)	0.297	130 (78.31)	36 (21.69)	0.400
			-	232 (56.31)	156 (37.86)	24 (5.83)		620 (75.24)	204 (24.76)	
		Drug resistance	+	41 (55.41)	31 (41.89)	2 (2.7)	0.298	113 (76.35)	35 (23.65)	0.855
			-	244 (57.96)	149 (35.39)	28 (6.65)		637 (75.65)	205 (24.35)	
		DILI	+	46 (67.65)	20 (29.41)	2 (2.94)	0.157	112 (82.35)	24 (17.65)	0.053
			-	239 (55.97)	160 (37.47)	28 (6.56)		638 (74.71)	216 (25.29)	
	Pulmonary infection	+	36 (59.02)	20 (32.79)	5 (8.2)	0.672	92 (75.41)	30 (24.59)	0.924	
		-	249 (57.37)	160 (36.87)	25 (5.76)		658 (75.81)	210 (24.19)		
	Hypoproteinemia	+	26 (63.41)	12 (29.27)	3 (7.32)	0.607	64 (78.05)	18 (21.95)	0.613	
		-	259 (57.05)	168 (37)	27 (5.95)		686 (75.55)	222 (24.45)		
	Sputum smear-positive	+	80 (57.55)	56 (40.29)	3 (2.16)	0.085	216 (77.7)	62 (22.3)	0.294	
		-	176 (56.23)	114 (36.42)	23 (7.35)		466 (74.44)	160 (25.56)		
rs2971205	A/G	Fever	+	52 (62.65)	25 (30.12)	6 (7.23)	0.732	129 (77.71)	37 (22.29)	0.899
			-	254 (61.65)	136 (33.01)	22 (5.34)		644 (78.16)	180 (21.84)	
		Drug resistance	+	45 (60.81)	25 (33.78)	4 (5.41)	0.967	115 (77.7)	33 (22.3)	0.904
			-	261 (62)	136 (32.3)	24 (5.7)		658 (78.15)	184 (21.85)	
		DILI	+	41 (60.29)	24 (35.29)	3 (4.41)	0.806	106 (77.94)	30 (22.06)	0.966
			-	265 (62.06)	137 (32.08)	25 (5.85)		667 (78.1)	187 (21.9)	
	Pulmonary infection	+	34 (55.74)	21 (34.43)	6 (9.84)	0.267	89 (72.95)	33 (27.05)	0.144	
		-	272 (62.67)	140 (32.26)	22 (5.07)		684 (78.8)	184 (21.2)		
	Hypoproteinemia	+	24 (58.54)	15 (36.59)	2 (4.88)	0.838	63 (76.83)	19 (23.17)	0.775	
		-	282 (62.11)	146 (32.16)	26 (5.73)		710 (78.19)	198 (21.81)		
	Sputum smear-positive	+	83 (59.71)	46 (33.09)	10 (7.19)	0.680	212 (76.26)	66 (23.74)	0.573	
		-	191 (61.02)	106 (33.87)	16 (5.11)		488 (77.96)	138 (22.04)		
rs75368388	T/A	fever	+	68 (81.93)	15 (18.07)	0 (0)	0.287	151 (90.96)	15 (9.04)	0.252
			-	360 (87.38)	50 (12.14)	2 (0.49)		770 (93.45)	54 (6.55)	
		Drug resistance	+	60 (81.08)	13 (17.57)	1 (1.35)	0.171	133 (89.86)	15 (10.14)	0.101
			-	368 (87.41)	52 (12.35)	1 (0.24)		788 (93.59)	54 (6.41)	
		DILI	+	63 (92.65)	5 (7.35)	0 (0)	0.263	131 (96.32)	5 (3.68)	0.104
			-	365 (85.48)	60 (14.05)	2 (0.47)		790 (92.51)	64 (7.49)	
	Pulmonary infection	+	53 (86.89)	8 (13.11)	0 (0)	0.868	114 (93.44)	8 (6.56)	0.849	
		-	375 (86.41)	57 (13.13)	2 (0.46)		807 (92.97)	61 (7.03)		
	Hypoproteinemia	+	36 (87.8)	5 (12.2)	0 (0)	0.896	77 (93.9)	5 (6.1)	0.746	
		-	392 (86.34)	60 (13.22)	2 (0.44)		844 (92.95)	64 (7.05)		
	Sputum smear-positive	+	118 (84.89)	21 (15.11)	0 (0)	0.450	257 (92.45)	21 (7.55)	0.645	
		-	273 (87.22)	38 (12.14)	2 (0.64)		584 (93.29)	42 (6.71)		

(Continued)

Table 3 (Continued).

SNP	Allele	Clinical Features	Group	Genotype			Pvalue	Allele		P-value
	(Mm)			MM	Mm	mm		M	m	
rs9288953	T/C	Fever	+	23 (27.71)	37 (44.58)	23 (27.71)	0.314	83 (50)	83 (50)	0.732
			-	102 (24.76)	220 (53.4)	90 (21.84)		424 (51.46)	400 (48.54)	
		Drug resistance	+	13 (17.57)	38 (51.35)	23 (31.08)	0.098	64 (43.24)	84 (56.76)	0.035
			-	112 (26.6)	219 (52.02)	90 (21.38)		443 (52.61)	399 (47.39)	
		DILI	+	22 (32.35)	36 (52.94)	10 (14.71)	0.145	80 (58.82)	56 (41.18)	0.849
			-	103 (24.12)	221 (51.76)	103 (24.12)		427 (50)	427 (50)	
		Pulmonary infection	+	18 (29.51)	28 (45.9)	15 (24.59)	0.583	64 (52.46)	58 (47.54)	0.769
			-	107 (24.65)	229 (52.76)	98 (22.58)		443 (51.04)	425 (48.96)	
		Hypoproteinemia	+	10 (24.39)	21 (51.22)	10 (24.39)	0.968	41 (50)	41 (50)	0.819
			-	115 (25.33)	236 (51.98)	103 (22.69)		466 (51.32)	442 (48.68)	
		Sputum smear-positive	+	32 (23.02)	77 (55.4)	30 (21.58)	0.625	141 (50.72)	137 (49.28)	0.947
			-	79 (25.24)	158 (50.48)	76 (24.28)		316 (50.48)	310 (49.52)	

Note: +, with; -, without; part of the study subjects of data missing.

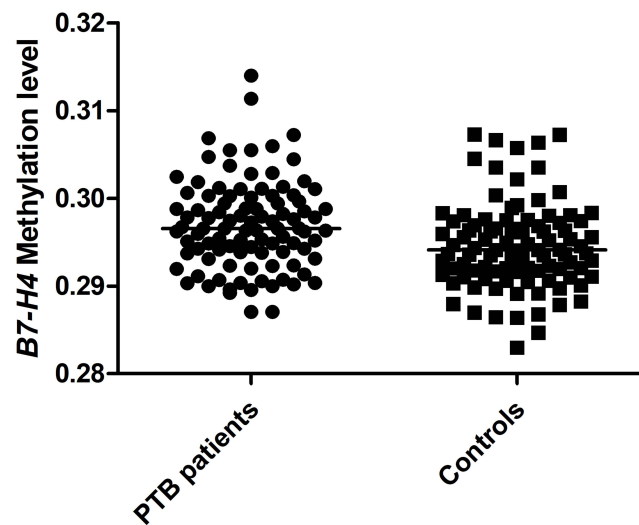


Figure 1 The methylation levels of *B7-H4* genes in PTB patients and controls.

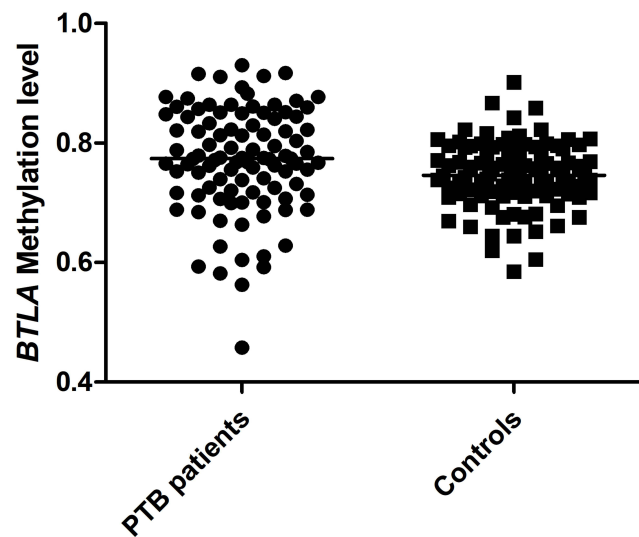


Figure 2 The methylation levels of *BTLA* genes in PTB patients and controls.

with the risk of breast cancer.²⁶ The results by Chen et al indicated that two polymorphisms (rs10801935, rs3738414) in *B7-H4* gene were significantly associated with AS susceptibility, and TAG haplotype was a risk factor for AS by haplotype analysis.²⁰ Therefore, we speculated that *B7-H4* gene polymorphism might also be related to the susceptibility to PTB, and analyzed the association between five SNPs (rs10754339, rs10801935, rs10923223, rs1937956, rs3738414) in *B7-H4* gene with PTB risk. The rs3738414 GA genotype was found to increase susceptibility to PTB, but this association was not statistically significant after Bonferroni correction. Furthermore, AATTG haplotype in the *B7-H4* gene might be associated with an increased risk of PTB. This suggested that *B7-H4* gene variation might be involved in the pathogenesis of PTB, but further verification was needed.

As a newly discovered inhibitory receptor, *BTLA* exerted important roles in suppressing the overreactive immune response and could play immunosuppressive effects on the host by activating B cells and T cells.²⁷ Recent evidences described the important function of *BTLA* in many diseases, including inflammatory disorder, autoimmune diseases and PTB.^{23,28} Previous studies regarding the roles of *BTLA* on PTB mainly focused on its expression level in several specific cell.^{18,23} Wang et al revealed the effect of *BTLA* in CD11c antigen presenting cells (APCs) in active PTB patients, and

Table 4 Association Between *B7-H4*, *BTLA* Methylation Levels and Clinical Characteristics of PTB Patients

Group	Group	N	<i>B7-H4</i> Methylation level	P-value	<i>BTLA</i> Methylation Level	P-value
Fever	+	16	0.2972 (0.2955–0.3011)	0.414	0.7786 (0.6955–0.8511)	0.962
	–	82	0.2966 (0.2931–0.3001)		0.7735 (0.7193–0.8433)	
Drug resistance	+	12	0.2946 (0.2906–0.2966)	0.061	0.8001 (0.7629–0.8413)	0.324
	–	86	0.2973 (0.2941–0.3003)		0.7721 (0.7131–0.8433)	
DILI	+	13	0.2988 (0.2921–0.3016)	0.322	0.7670 (0.6938–0.8455)	0.626
	–	85	0.2965 (0.2938–0.2995)		0.7739 (0.7251–0.8434)	
Pulmonary infection	+	12	0.2964 (0.2917–0.2996)	0.991	0.7243 (0.6719–0.8316)	0.165
	–	86	0.2966 (0.2938–0.3003)		0.7742 (0.7253–0.8446)	
Hypoproteinemia	+	4	0.3023 (0.2951–0.3122)	0.148	0.8636 (0.7611–0.9031)	0.090
	–	94	0.2965 (0.2936–0.3001)		0.7729 (0.7156–0.8345)	
Sputum smear-positive ^a	+	34	0.2966 (0.2929–0.2990)	0.477	0.7864 (0.7298–0.8494)	0.312
	–	54	0.2973 (0.2942–0.3011)		0.7723 (0.7228–0.8241)	

Note: +, with; –, without. ^aPart of the study subjects of data missing.

Table 5 Association between *B7-H4*, *BTLA* genes genotypes on their methylation levels

<i>B7-H4</i> SNP	Genotype	Number	<i>B7-H4</i> Methylation Level	P-value
rs10754339	GG	3	0.3055 (0.2975–0.3140)	0.040
	GA	13	0.2945 (0.2903–0.2985)	
	AA	82	0.2966 (0.2939–0.3003)	
rs10801935	CC	5	0.2964 (0.2896–0.3106)	0.867
	CA	29	0.2975 (0.2923–0.3012)	
	AA	64	0.2966 (0.2940–0.2993)	
rs10923223	CC	17	0.2988 (0.2963–0.3022)	0.120
	CT	45	0.2962 (0.2923–0.2997)	
	TT	36	0.2964 (0.2934–0.2993)	
rs1937956	AA	3	0.3055 (0.2975–0.3140)	0.129
	AT	19	0.2965 (0.2907–0.2994)	
	TT	76	0.2964 (0.2938–0.3002)	
rs3738414	AA	5	0.2966 (0.2928–0.3005)	0.209
	GA	46	0.2955 (0.2922–0.2990)	
	GG	47	0.2976 (0.2945–0.3006)	
<i>BTLA</i> SNP	Genotype	Number	<i>BTLA</i> methylation level	P value
rs1982809	AA	6	0.7455 (0.6757–0.7600)	0.126
	AG	37	0.7755 (0.7465–0.8548)	
	GG	55	0.7746 (0.7125–0.8432)	
rs2971205	GG	3	0.7555 (0.7163–0.8590)	0.959
	AG	31	0.7745 (0.7392–0.8293)	
	AA	64	0.7734 (0.7063–0.8468)	
rs75368388	AA	0	–	0.127
	TA	15	0.7524 (0.6882–0.7915)	
	TT	83	0.7746 (0.7247–0.8479)	
rs9288953	CC	20	0.7615 (0.7220–0.7940)	0.429
	CT	59	0.7739 (0.7203–0.8293)	
	TT	19	0.8223 (0.6993–0.8639)	

found that *BTLA*-expressing CD11c APCs showed a stronger ability to stimulate T cell proliferation.¹⁸ Another study demonstrated that the expression of CD11c⁺ *BTLA* was positively related to CRP, which might be correlated with inflammatory changes in PTB patients.²⁹ The present study mainly focused on studying the association of four SNPs (rs1982809, rs2971205, rs75368388, rs9288953) in *BTLA* gene with PTB risk, while no significant association was found between these SNPs and the risk of PTB. Contrary to our study, previous studies displayed significant correlation between *BTLA* gene polymorphisms and cancer, autoimmune diseases. Andrzejczak et al found that rs1982809 variant was significantly related to non-small cell lung cancer risk.³⁰ Karabon et al reported that rs1982809, rs9288953 variants would affect the susceptibility to chronic lymphocytic leukemia.³¹ It was worth noting that rs9288953 variant was found to be significantly associated with the development of drug resistance among PTB patients. Therefore, *BTLA* gene polymorphism could be used as a potential index to determine the clinical classification of PTB patients.

In addition to DNA sequences, important genetic information was also present in epigenetic variations.³² Gene polymorphism and epigenetic mechanisms might interact and together influenced the biological function and disease progression.³³ Therefore, the regulatory role of abnormal DNA methylation, which was considered as a common epigenetic trait, of certain key genes in human diseases had been reported.³⁴ DNA methylation had become an irreplaceable way to explore the pathogenesis of PTB. The results of Wang et al showed that abnormal methylation status of several key genes in the vitamin D metabolic pathway was closely connected with the risk and prognosis of PTB.¹² Considering the potential significance of *B7-H4* and *BTLA* genes polymorphisms in PTB development, this study further analyzed the relationship among their methylation levels and PTB risk. Here, the present results demonstrated that the methylation levels of *B7-H4* and *BTLA* genes were significantly increased in PTB patients. Interestingly, we found that *B7-H4* methylation level was affected rs10754339 variants among PTB patients. This implied that abnormal methylation levels of *B7-H4* and *BTLA* genes were involved in the pathogenesis of PTB, and there might be an interaction between *B7-H4* gene polymorphism and its methylation in PTB patients. The above findings provided important clues to reveal the occurrence of PTB, while relevant results still needed to be further verified.

Some limitations of this study should be noted. First, our study only screened several tagSNPs in *B7-H4* and *BTLA* genes, and some SNPs with important functions might be missed. Second, we compared methylation levels of *B7-H4* and *BTLA* genes between PTB patients and controls in a cross-sectional study, and did not analyze their changes levels over the course of PTB development. Third, our study did not exclude the possible influence of environmental factors and ethnic background on the results.

In summary, *B7-H4*, *BTLA* genes polymorphism might not be affected PTB susceptibility in the Chinese population, while the abnormal methylation levels of *B7-H4*, *BTLA* genes were associated with PTB risk. These results firstly revealed the roles of *B7-H4*, *BTLA* genes methylation in the development of PTB, which was important for PTB control and prevention. Moreover, the further studies regarding the mechanism of *B7-H4*, *BTLA* genes variation on PTB development was still warranted in future.

Ethics Approval and Consent to Participate

This study was approved by the Ethical Committee of Anhui Chest Hospital (K2023-011). All the study subjects provided informed consent to participate in this study.

Data Sharing Statement

The data generated and analyzed by this study are available from the corresponding author on reasonable request.

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Disclosure

Xue-Qian Cai and Qian Huang are co-first authors for this study. The authors report no conflicts of interest in this work.

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