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Polymorphisms of the *BCL2* gene associated with susceptibility to tuberculosis

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ABSTRACT

Although tuberculosis (TB) is a serious public health concern, we still don't understand why only 10% of people infected will develop the disease. Apoptosis plays a role in the interaction of *Mycobacterium tuberculosis (Mtb)* with the human host and it may be modified by subtle alterations in the B-cell lymphoma 2 (*BCL2*) gene, an anti-apoptotic regulatory element. Therefore, we investigated whether there is an association between *BCL2* polymorphisms and susceptibility to TB by analyzing 130 TB cases, 108 subjects with latent TB infection (LTBI), and 163 healthy controls (HC). Logistic regression was used to calculate odds ratios (ORs) and 95% confidential intervals (95% CIs) for possible associations between single nucleotide polymorphisms (SNPs) in *BCL2* and the risk of tuberculosis. We found that the G allele of rs80030866 (OR=0.62, 95%CI:0.42-0.91, *P*=0.015), and also the G allele of rs9955190 (OR=0.58, 95%CI:0.38-0.88, *P*=0.011) were less frequent in the TB group compared with the LTBI group. In addition, individuals with rs2551402 CC genotype were more likely to have LTBI than those with AA genotype (OR=2.166, 95%CI:1.046-4.484, *P*=0.037). Our study suggests that *BCL2* gene polymorphisms may be correlated with susceptibility to both TB and LTBI.

KEYWORDS: Tuberculosis. Susceptibility. Latent tuberculosis infection. *BCL2*. Polymorphism.

INTRODUCTION

Tuberculosis (TB) is a transmittable infectious disease caused by *Mycobacterium tuberculosis (Mtb)* that primarily targets the lungs. Globally, an estimate of 9.9 million people have TB and 1.3 million TB deaths among HIV-negative people were reported in 2020[1], confirming that it is still a leading public health problem. As a consequence of disruptions in TB control caused by the COVID-19 pandemic, deaths due to TB in high-burden settings may increase by up to 20% over the next 5 years[2].

Individuals who are in close contact with active TB cases have a higher risk of developing latent TB infection (LTBI). People with LTBI show a persistent immune response to *Mtb* antigens, but have no evidence to clinically manifest TB disease[3]. However, in a small percentage of individuals with LTBI, the immune system will fail to control the infection at some point in their lives and they will develop active TB, so they represent a potential reservoir for new TB cases[4]. It is estimated that about one-fourth of the world's population is infected with *Mtb*[5] but only 5%-10% of infected individuals will develop TB disease[6]. The immunological mechanisms that either restrict the infection or allow it to progress to active disease remain poorly understood, but host genetic factors have been suspected to play an important role[7].

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Studies have linked TB disease susceptibility to polymorphisms in several candidate target genes[8], but many of these associations have been difficult to ascertain. Genome-wide association (GWAS) studies have also been used to find loci associated with susceptibility to TB, but surprisingly, GWAS did not find any association with the loci identified in the candidate gene studies[9]. Therefore, the specific genetic elements that influence the risk of TB infection and disease are still largely unknown. Nevertheless, if TB risk-associated genes could be identified, they would help to clarify the pathogenesis of this disease and enable personalized treatment based upon an individual's risk of infection and progression to disease.

Previous studies have shown that apoptosis of macrophages[10] and T cells[11] plays a vital role in host defense against *Mtb* and TB pathogenesis. The B-cell lymphoma 2 (*BCL2*) gene functions as an antiapoptotic regulatory element and its down-regulation correlate with an increased risk of TB disease progression[12]. Therefore, we decided to investigate the possible role of *BCL2* in TB genetic susceptibility through a case-control study of the association of *BCL2* gene polymorphisms with TB/LTBI risk in the Chinese Han population.

MATERIALS AND METHODS

Study participants

The study enrolled 130 pulmonary TB patients and 279 close contacts of individuals with sputum smear or culture-positive TB. Eight out of 279 contacts were ruled out in the quality control of genotyping, leaving 271 cases for the final analysis. All participants were recruited from the Chinese Han population visiting the Shenzhen Nanshan Center for Chronic Disease Control (22° N 113° E) from May 2017 to July 2018 and all were vaccinated with Bacillus Calmette-Guerin (BCG) in their infancy. TB patients were newly diagnosed by clinical specialists according to microbiological criteria (positive sputum smear or cultures), clinical manifestations, and radiology (chest X-rays or computed tomography scans). To detect LTBI, both interferon-gamma release assays (IGRA) and the Mantoux tuberculin skin test (TST) are recommended by WHO[13,14]. In our study, we used IGRA to separate the contacts into those with LTBI and the uninfected healthy controls (HC). LTBI were IGRA positive, while HC had negative IGRA tests. LTBI and HC individuals had no TB-related symptoms and negative sputum for acid-fast bacilli by microscopy. Participants with cancer, concomitant chronic obstructive pulmonary disease, HIV infection, hepatitis B virus (HBV) infection, HCV infection, or immune-mediated disorders were excluded.

Extraction of genomic DNA

Blood samples were collected from all participants in EDTA tubes, followed by genomic DNA extraction using QIAamp® DNA blood mini kit (QIAGEN Inc., Hilden, Germany), by the manufacturer's instructions. The extracted DNA was diluted to a working concentration of 50 ng/μL for further genotyping.

SNP selection and genotyping

The SNPs in the *BCL2* gene were selected from the 1000 Genomes Project based on tag SNPs with a minor allele frequency ≥ 0.05 and $r^2 > 0.8$, using Haploview software (version 4.2, Broad Institute of MIT and Harvard, Cambridge, MA, USA). These tag SNPs were annotated for their location and predictive functions[15]. Study subjects were genotyped using the Illumina HumanOmniZhongHua-8 BeadChip. The analysis was repeated on 5% of the samples to evaluate the reproducibility and quality of genotyping. To exclude possible genotyping errors, we generated genotype cluster plots from the selected SNPs and visually inspected them using GenomeStudio software (version 2.0, Illumina, San Diego, CA, USA).

Ethics approval

Signed informed consent was obtained from all participants and ethics approval was granted by the Ethics Review Committee of the Shenzhen Nanshan Center for Chronic Disease Control (ll20170018).

Statistical analyses

T-test and χ^2 test were used to compare age and gender distributions between the patients and controls. Deviation from Hardy–Weinberg equilibrium (HWE) was assessed using the SNPassoc package of R (version 4.0.3, R Project for Statistical Computing, Vienna, Austria). The differences in allele frequencies between the three groups of subjects were performed with the χ^2 test. Logistic regression analysis, under genotypes and genetic models (dominant, recessive, and additive model)[16], adjusting for age and gender, was used to assess possible associations between *BCL2* SNPs and TB susceptibility, and to calculate 95% confidence intervals (95% CIs), odds ratios (ORs), and *P* values. Subgroup analysis was performed by age, gender, sputum smear status, pulmonary cavities, and course of treatment, obtained from the clinical records. Haploview (version 4.2) was used to assess linkage disequilibrium (LD) of

the SNP sites and haplotype analysis was performed using the SHEsis online software platform. Power calculations were performed with PASS software (version 11.0, NCSS, Kaysville, Utah, USA). *P* values were from two-tailed tests and statistical significance was set at $P < 0.05$. *P* values were then adjusted by Bonferroni correction for the ten polymorphisms examined so that a *P*-value < 0.005 (0.05/10) was considered statistically significant. The genotyping data were processed using GenomeStudio 2.0 and PLINK (version 1.90) for Windows, and statistical analyses were performed using SPSS Statistics (version 22.0, IBM, Chicago, USA).

RESULTS

Characteristics of the enrolled subjects

Of the total 409 subjects enrolled in the study, 401(98.04%) were successfully genotyped, including 130 TB cases, 108 LTBI individuals, and 163 HCs. The characteristics of the three groups are shown in Table 1. There was no significant difference in the gender distribution, but the TB patients were significantly younger than the LTBI and HC cohorts (*P*<0.05).

Association of *BCL2* SNPs and TB / LTBI susceptibility

The reproducibility of the genotyping was 100% according to the duplicate genotyping results. The distribution of the genotypes of 10 *BCL2* SNPs among 163 HCs fully met the Hardy Weinberg equilibrium (*P*>0.05). We found that five SNPs were significantly associated with TB or LTBI (rs80030866, rs3744933, rs9955190, rs1801018 and rs2551402, all *P*<0.05) (Table 2). However, the associations did not reach the statistical significance of 0.005 stipulated by the Bonferroni correction for 10 separate comparisons.

For rs80030866 (A>G), the frequency of the G allele was lower in the TB group (OR=0.62, 95%CI:0.42-0.91, *P*=0.015) compared with the LTBI group (Table 2).

For rs3744933 (C>A), the frequency of the A allele was higher in the TB group (OR=1.81, 95%CI:1.05-3.12, *P*=0.031) when compared to the HC group, and the heterozygous AC genotype was more frequent in the TB group than the CC genotype (OR=2.014, 95%CI:1.105-3.672, *P*=0.022) (Table 2). Compared to the HC group, the association of TB with the rs3744933 A allele remained significant using either dominant (OR=1.986, 95%CI: 1.099-3.587, *P*=0.023) or additive (OR=0.498, 95%CI: 0.273-0.907, *P*=0.023) genetic models (Table 3).

For rs9955190 (A>G), the frequency of the G allele was lower in the TB group when compared to the LTBI group (OR=0.58, 95%CI:0.38-0.88, *P*=0.011), and the TB group had significantly fewer GG genotypes than AA genotypes (OR=0.165, 95%CI:0.033-0.821, *P*=0.028) (Table 2).

For rs1801018 (A>G), the frequency of the G allele was higher in the LTBI group when compared to the HC group (OR=1.97, 95%CI:1.09-3.57, *P*=0.022) (Table 2).

For rs2551402 (A>C), the CC genotype was more common than the AA genotype in the LTBI group when compared to the HC group (OR=2.166, 95%CI:1.046-4.484,

Table 1 - Demographic and clinical characteristics of the study groups.

	TB (*n*=130)	LTBI (*n*=108)	HC (*n*=163)	TB *vs.* LTBI *P*-value	TB *vs.* HC *P*-value	LTBI *vs.* HC *P*-value
Age, mean ±S.D.	31.18±10.85	40.09±12.42	34.15±12.57	**<0.001**	**0.034**	**<0.001**
Gender, *n* (%)				0.286	0.415	0.729
Male	74(56.9)	54(50.0)	85(52.1)			
Female	56(43.1)	54(50.0)	78(47.9)			
Sputum smear status, *n* (%)						
Smear-positive	53(40.8)					
Smear-negative *(Culture-positive)*	77(59.2)					
Pulmonary cavity, *n* (%)						
Present	43(33.1)					
Absent	87(66.9)					
Course of treatment, *n* (%)						
> 6 months	49(38.3)					
≤ 6 months	79(61.7)					

Table 2 - The positive results of genetic association analysis in three groups.

SNPs	Genotypes*/ Alleles	TB	LTBI	HC	TB vs. LTBI		TB vs. HC		LTBI vs. HC	
		N (%)	N (%)	N (%)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)
rs80030866	AA	65 (50.4)	42 (38.9)	70 (43.0)	Rf					
A>G	GA	55 (42.6)	48 (44.4)	75 (46.0)	0.531	0.837 (0.512-1.369)	0.479	0.837 (0.512-1.369)	0.805	1.07 (0.623-1.838)
	GG	9 (7.0)	18 (16.7)	18 (11.0)	0.077	0.575 (0.238-1.388)	0.218	0.575 (0.238-1.388)	0.460	1.344 (0.613-2.949)
	A	185 (71.7)	132 (61.1)	215 (66.0)						
	G	73 (28.3)	84 (38.9)	111 (34.0)	**0.015**	0.62 (0.42-0.91)	0.137	0.76 (0.54-1.09)	0.250	1.23 (0.86-1.76)
rs3744933	CC	97 (74.6)	92 (85.2)	139 (85.3)	Rf					
C>A	AC	32 (24.6)	15 (13.9)	23 (14.1)	0.063	1.979 (0.965-4.058)	**0.02**	2.014 (1.105-3.672)	0.865	1.064 (0.519-2.184)
	AA	1 (0.8)	1 (0.9)	1 (0.6)	——	——	——	——	——	——
	C	226 (86.9)	199 (92.1)	301 (92.3)						
	A	34 (13.1)	17 (7.9)	25 (7.7)	0.067	1.76 (0.95-3.25)	**0.03**	1.81(1.05-3.12)	0.931	1.03(0.54-1.95)
rs9955190	AA	81 (62.3)	57 (52.8)	89 (54.6)	Rf					
A>G	GA	47 (36.2)	38 (35.2)	65 (39.9)	0.463	1.254 (0.685-2.295)	0.513	0.85 (0.521-1.385)	0.467	0.819 (0.478-1.403)
	GG	2 (4.5)	13 (12.0)	9 (5.5)	**0.028**	0.165 (0.033-0.821)	0.098	0.265 (0.055-1.278)	0.279	1.684 (0.656-4.324)
	A	209 (80.4)	152 (70.4)	243 (74.5)						
	G	51 (19.6)	64 (29.6)	83 (25.5)	**0.011**	0.58 (0.38-0.88)	0.094	0.71 (0.48-1.06)	0.285	1.23 (0.84-1.81)
rs1801018	AA	107 (82.3)	85 (78.7)	141 (86.5)	Rf					
A>G	GA	22 (16.9)	19 (17.6)	22 (13.5)	0.786	1.108 (0.529-2.317)	0.368	1.346 (0.704-2.573)	0.358	1.382 (0.693-2.754)
	GG	1 (0.8)	4 (3.7)	0 (0.0)	——	——	——	——	——	——
	A	236 (90.8)	189 (87.5)	304 (93.3)						
	G	24 (9.2)	27 (12.5)	22 (6.7)	0.201	0.68 (0.38-1.23)	0.336	1.35 (0.73-2.48)	**0.022**	1.97(1.09-3.57)
rs2551402	AA	45 (34.6)	34 (31.5)	65 (39.9)	Rf					
A>C	CA	63 (48.5)	50 (46.3)	75 (46.0)	0.733	1.114 (0.598-2.079)	0.454	1.215 (0.729-2.025)	0.533	1.196(0.681-2.103)
	CC	22 (16.9)	24 (22.2)	23 (14.1)	0.422	0.725 (0.331-1.59)	0.430	1.328 (0.656-2.686)	**0.037**	2.166 (1.046-4.484)
	A	153 (58.8)	118 (54.6)	205 (62.9)						
	C	107 (41.2)	98 (45.4)	121 (37.1)	0.355	0.84 (0.58-1.21)	0.319	1.18 (0.85-1.65)	0.055	1.41 (0.99-2.00)

— = all those frequencies <0.03 will be ignored in analysis; *Adjusted for age and gender.

Table 3 - The positive results of the association between *BCL2* genotypes and LTBI/TB based on genetic model analysis.

SNPs	Models		Groups, N (%)			TB vs. LTBI		TB vs. HC		LTBI vs. HC	
			TB	LTBI	HC	*P**	OR (95%CI)*	*P**	OR (95%CI)*	*P**	OR (95%CI)*
rs3744933 C>A	Dominant	AA+AC	33 (25.4)	16 (14.8)	24 (14.7)	0.078	1.880 (0.932-3.793)	**0.023**	**1.986 (1.099-3.587)**	0.795	1.097 (0.544-2.214)
		CC	97 (74.6)	92 (85.2)	139 (85.3)	Rf					
	Recessive	AA	1 (0.8)	1 (0.9)	1 (0.6)	——	——	——	——	——	——
		AC+CC	129 (99.2)	107 (99.1)	162 (99.4)						
	Additive	AA+CC	98 (75.4)	93 (86.1)	140 (85.9)	0.060	0.502 (0.245-1.029)	**0.023**	**0.498 (0.273-0.907)**	0.878	0.945 (0.461-1.939)
		AC	32 (24.6)	15 (13.9)	23 (14.1)	Rf					
rs2551402 A>C	Dominant	CC+CA	85 (65.4)	74 (68.5)	98 (60.1)	0.954	0.983 (0.550-1.759)	0.379	1.242 (0.767-2.012)	0.199	1.412 (0.834-2.388)
		AA	45 (34.6)	34 (31.5)	65 (39.9)	Rf					
	Recessive	CC	22 (16.9)	24 (22.2)	23 (14.1)	0.281	0.681 (0.339-1.369)	0.595	1.191 (0.626-2.266)	**0.044**	**1.960 (1.018-3.772)**
		CA+AA	108 (83.1)	84 (77.8)	140 (85.9)	Rf					
	Additive	CC+AA	67 (51.5)	58 (53.7)	88 (54.0)	0.427	0.799 (0.459-1.390)	0.638	0.894 (0.561-1.425)	0.770	1.078 (0.652-1.784)
		CA	63 (48.5)	50 (46.3)	75 (46.0)	Rf					

— = all those frequencies <0.03 will be ignored in analysis; *Adjusted for age and gender.

P=0.037) (Table 2), and significant in a recessive model (OR=1.960, 95%CI: 1.018-3.772, *P*=0.044) (Table 3).

Power analysis

We calculated the power of the study using ORs of 2.0, 3.0, and 4.0 to determine whether the study sample size was adequate for the detection of associations[17]. The results indicated that 8 of the analyzed SNPs had a power approaching 80% to find an association with an OR ≥ 2 (Table 4). However, for the rs3744933 and rs1801018 loci, the statistical power was insufficient, and larger sample sizes would be needed to explore possible associations with tuberculosis.

Subgroup analyses

Subgroup analyses were performed on rs80030866 and rs9955190 based on age, gender, sputum smear status, pulmonary cavities, and course of treatment. We found significant differences in the allelic frequencies of rs80030866 (OR=0.545, 95%CI:0.318-0.933, *P*=0.026) and rs9955190 (OR=0.530, 95%CI:0.299-0.942, *P*=0.029) between TB and LTBI in males (Table 5). The rs80030866*GA+GG was less prevalent than the rs80030866*AA in patients requiring treatment for more than 6 months compared to patients treated for the standard 6 months or less (OR=0.461, 95%CI: 0.221-0.959, *P*=0.037) (Table 5).

Association between *BCL2* haplotypes and TB / LTBI susceptibility

Two *BCL2* haplotypes were constructed with Haploview (version 4.2), and relatively strong LDs were observed between rs80030866 and rs9955190 (pairwise D'=0.88, r^2=0.51) and between rs12458289 and rs2551402 (pairwise D'=1.00, r^2=0.56) (Figure 1). The rs80030866-rs9955190 AA haplotype was more frequent in the TB group than in the LTBI group (*P*=0.018193), while the GG haplotype was less frequent in the TB group than in either the LTBI group (*P*=0.003391) or the HC group (*P*=0.034859) (Table 6). No significant associations with TB were observed for the rs12458289-rs2551402 haplotypes.

DISCUSSION

In this study, we focused on the relationship between 10 SNPs in the *BCL2* gene and two states of TB infection – latent infection (LTBI) and active disease (TB). We compared the three study groups – HC, LTBI, and TB – for the frequency of alleles in the 10 SNPs to try to identify genetic markers associated with either LTBI or TB. We found that both the G allele of rs80030866 and the G allele of rs9955190 were more common in the LTBI group when compared to those with active TB. In addition, LTBI individuals were more likely to have the rs2551402 CC genotype than the AA genotype. Although several of these associations had *P* values < 0.05, they did not reach

Table 4 - Power of the study with different odds ratios (OR) in an allelic model.

SNPs	MAF	Power in TB vs. LTBI			Power in TB vs. HC			Power in LTBI vs. HC		
		OR=2	OR=3	OR=4	OR=2	OR=3	OR=4	OR=2	OR=3	OR=4
rs1564483	0.3998	0.7531	0.987	0.9995	0.8345	0.9964	0.9999	0.7936	0.9927	0.9998
rs956572	0.4643	0.7499	0.9841	0.9991	0.8304	0.9954	0.9999	0.7883	0.9909	0.9997
rs12454712	0.4583	0.7508	0.9845	0.9992	0.8313	0.9955	0.9999	0.7894	0.9912	0.9997
rs80030866	0.3383	**0.7414**	0.9872	0.9996	**0.8257**	0.9964	1.000	**0.7848**	0.9927	0.9999
rs3744933	0.1349	**0.5201**	0.9252	0.9942	**0.6213**	0.9656	0.9986	**0.583**	0.9484	0.9967
rs9955190	0.2669	**0.7045**	0.9832	0.9995	**0.7947**	0.9948	0.9999	**0.7531**	0.99	0.9998
rs12458289	0.2907	0.7202	0.9852	0.9996	0.8081	0.9956	1.000	0.7668	0.9913	0.9998
rs949037	0.3065	0.7286	0.9861	0.9996	0.8151	0.996	1.000	0.774	0.992	0.9998
rs1801018	0.0923	**0.4058**	0.8376	0.9746	**0.5014**	0.9089	0.991	**0.4708**	0.8807	0.9839
rs2551402	0.4177	**0.7537**	0.9865	0.9994	**0.8347**	0.9962	0.9999	**0.7935**	0.9924	0.9998

SNP = single nucleotide polymorphism; MAF = minor allele frequency.

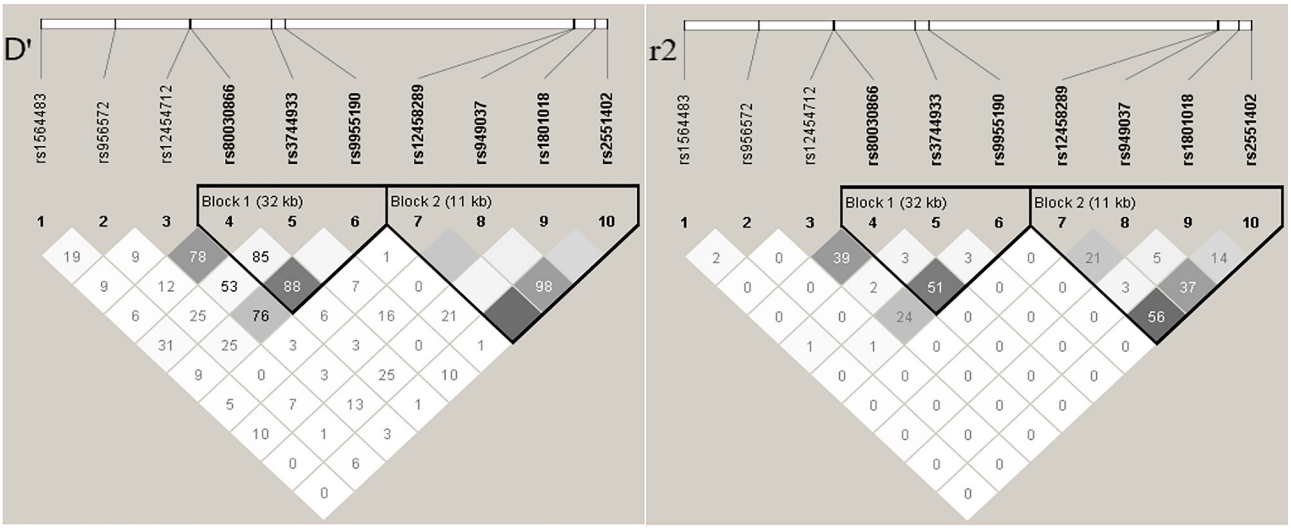

Figure 1 - Plot of linkage disequilibrium (LD) by Haploview (version 4.2). Pair-wise LD between all pairs of SNPs in *BCL2* was evaluated by D' and r^2 statistics. The D' and r^2 values (%) are presented in the squares.

the more stringent 0.005 level of statistical significance stipulated by the Bonferroni correction for comparing 10 different polymorphisms. In a study with a larger cohort or fewer polymorphisms tested, the associations would likely reach significance.

The *BCL2* gene, located on human chromosome 18, consists of three exons and two introns. *BCL2* interacts with other pro and anti-apoptotic BCL2 family proteins to regulate mitochondrial permeability and affect endogenous apoptosis, thereby altering the fate of cells. Studies using *in vitro*[18] and mouse models[19] have shown that the expression levels of *BCL2* in macrophages containing *Mtb* may affect the intracellular survival of the bacilli, and a population-based study suggested that the *BCL2* expression levels may predict the onset of active TB at a very early stage after infection[20]. Individuals with LTBI, as defined by a positive TST (tuberculin skin test) or IGRA test without clinical TB, can reactivate and develop active TB disease, but neither test can distinguish between LTBI and active TB nor predict which LTBI individuals will progress to active TB[21]. It is possible that *BCL2* expression levels change with the status of *Mtb* infection and thus may provide a way to distinguish between latent and active TB infection, or perhaps serve as an early marker to predict which LTBI individuals will develop active TB.

Lyu *et al.*[22] showed that *BCL2* variants may be associated with drug-induced liver injury associated with anti-tuberculous therapy. There is an interaction between *BCL2* and glutathione, an important regulator of lung inflammation, and restoration of *BCL2* expression leads to the replenishment of glutathione and a reduction in the

Table 5 - Association statistics of *BCL2* rs80030866 and rs9955190.

SNP	Group	Genotype, n (%)		*P*	OR (95%CI)	Allele, n (%)		*P*	OR (95%CI)
		AA	GA+GG			A	G		
rs80030866 A>G	Males TB	41 (55.4)	33 (44.6)	0.152	0.597 (0.294-1.212)	111 (75.0)	37 (25.0)	**0.026**	0.545 (0.318-0.933)
	Males LTBI	23 (42.6)	31 (57.4)			67 (62.0)	41 (38.0)		
	Females TB	24 (43.6)	31 (56.4)	0.367	0.701 (0.324-1.517)	74 (67.3)	36 (32.7)	0.276	0.735 (0.423-1.280)
	Females LTBI	19 (35.2)	35 (64.8)			65 (0.60)	43 (0.40)		
	≤ 31-year TB	45 (51.7)	42 (48.3)	0.062	0.444 (0.188-1.053)	124 (71.3)	50 (28.7)	0.221	0.684 (0.371-1.260)
	≤ 31-year LTBI	10 (32.3)	21 (67.7)			39 (62.9)	23 (37.1)		
	>32-year TB	20 (47.6)	22 (52.4)	0.524	0.782 (0.367-1.667)	61 (72.6)	23 (27.4)	0.059	0.575 (0.322-1.025)
	>32-year LTBI	32 (41.6)	45 (58.4)			93 (60.4)	61 (39.6)		
	Smear-positive	23 (43.4)	30 (56.6)	0.185	1.611 (0.795-3.267)	70 (66.0)	36 (34.0)	0.091	1.598 (0.925-2.761)
	Smear-negative	42 (55.3)	34 (44.7)			115 (75.7)	37 (24.3)		
	Pulmonary Cavity (Yes)	23 (53.5)	20 (46.5)	0.618	0.830 (0.399-1.728)	63 (73.3)	23 (26.7)	0.696	0.891 (0.499-1.591)
	Pulmonary Cavity (No)	42 (48.8)	44 (51.2)			122 (70.9)	50 (29.1)		
	Course of Treatment (>6 months)	30 (61.2)	19 (38.8)	**0.037**	0.461 (0.221-0.959)	74 (75.5)	24 (24.5)	0.211	0.695 (0.392-1.231)
	Course of Treatment (≤6 months)	32 (42.1)	44 (57.9)			105 (68.2)	49 (31.8)		
rs9955190 A>G	Males TB	46 (62.2)	28 (37.8)	0.337	0.706 (0.346-1.439)	119 (80.4)	29 (19.6)	**0.029**	0.530 (0.299-0.942)
	Males LTBI	29 (53.7)	25 (46.3)			74 (68.5)	34 (31.5)		
	Females TB	35 (62.5)	21 (37.5)	0.259	0.646 (0.302-1.382)	90 (0.80)	22 (0.20)	0.156	0.636 (0.339-1.191)
	Females LTBI	28 (51.9)	26 (48.1)			78 (72.2)	30 (27.8)		
	≤ 31-year TB	58 (66.7)	29 (33.3)	0.828	0.909 (0.385-2.149)	143 (82.2)	31 (17.8)	0.788	0.903 (0.431-1.893)
	≤ 31-year LTBI	20 (64.5)	11 (35.5)			50 (80.6)	12 (19.4)		
	>32-year TB	23 (53.5)	20 (46.5)	0.568	0.804 (0.381-1.699)	66 (76.7)	20 (23.3)	0.088	0.594 (0.326-1.085)
	>32-year LTBI	37 (48.1)	40 (51.9)			102 (66.2)	52 (33.8)		
	Smear-positive	32 (60.4)	21 (39.6)	0.706	1.148 (0.559-2.360)	84 (79.2)	22 (20.8)	0.701	1.129 (0.608-2.097)
	Smear-negative	49 (63.6)	28 (36.4)			125 (81.2)	29 (18.8)		
	Pulmonary Cavity (Yes)	27 (62.8)	16 (37.2)	0.936	0.970 (0.456-2.063)	70 (81.4)	16 (18.6)	0.773	0.908 (0.470-1.752)
	Pulmonary Cavity (No)	54 (62.1)	33 (37.9)			139 (79.9)	35 (20.1)		
	Course of Treatment (>6 months)	32 (65.3)	17 (34.7)	0.441	0.747 (0.356-1.570)	80 (81.6)	18 (18.4)	0.589	0.839 (0.442-1.590)
	Course of Treatment (≤6 months)	45 (58.4)	32 (41.6)			123 (78.8)	33 (21.2)		

Table 6 - Haplotype analysis of four SNPs of *BCL2*.

Haplotype	TB (freq)	LTBI (freq)	HC (freq)	TB vs. LTBI		TB vs. HC		LTBI vs. HC	
				P	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
rs80030866-rs9955190									
AA	178.08 (0.690)	128.71 (0.596)	210.54 (0.646)	**0.018193**	1.592 (1.081-2.344)	0.166053	1.286 (0.900-1.837)	0.242827	0.808 (0.565-1.156)
GA	29.92 (0.116)	23.29 (0.108)	32.46 (0.100)	0.744731	1.100 (0.619-1.956)	0.487492	1.205 (0.712-2.040)	0.751721	1.095 (0.624-1.923)
GG	43.08 (0.167)	60.71 (0.281)	78.54 (0.241)	**0.003391**	0.519 (0.333-0.808)	**0.034859**	0.641 (0.423-0.971)	0.288908	1.236 (0.835-1.828)
rs12458289-rs2551402									
AC	75.00 (0.288)	65.00 (0.301)	84.00 (0.258)	0.766381	0.942 (0.634-1.399)	0.404881	1.168 (0.810-1.683)	0.269410	1.240 (0.846-1.818)
CA	153.00 (0.588)	118.00 (0.546)	205.00 (0.629)	0.354998	1.188 (0.825-1.710)	0.319292	0.844 (0.604-1.179)	0.055233	0.711 (0.501-1.008)
CC	32.00 (0.123)	33.00 (0.153)	37.00 (0.114)	0.347447	0.778 (0.461-1.314)	0.720852	1.096 (0.662-1.815)	0.181882	1.408 (0.850-2.333)

Haplotypes with a frequency of <0.05 in the subjects were not evaluated.

levels of reactive oxygen species[23]. This suggests that *BCL2* could help limit the lung damage caused by TB and thus may warrant investigation as an adjunct therapy for the treatment of TB.

The rs80030866 and rs9955190 SNPs map to the intron region of the *BCL2* gene, and studies have shown that some introns encode small RNA transcripts that regulate gene expression and function[24]. HaploReg (version 4.1, Massachusetts Institute of Technology, Cambridge, MA, USA) indicates that both rs80030866 and rs9955190 are related to promoter and enhancer histone marks. Chromatin modification of histone marks is known to modify gene expression[25], so perhaps rs80030866 and rs9955190 regulate the expression of *BCL2*. The rs2551402 SNP is in strong linkage disequilibrium with rs1462129 (r^2=0.99), both of which are also located in the intron region of the *BCL2* gene. A large two-stage study reported that rs2551402 and rs1462129 play important roles in the regulation of apoptosis[26]. While these four SNPs are all located in the intron region of the *BCL2* gene, fine-mapping and functional studies are needed to identify the causal SNPs and elucidate the biological mechanisms underlying the associations we found with TB/LTBI risk.

The association of LTBI susceptibility with polymorphisms in several genes has been reported (e.g., in *SP110*[27]), but the relation of polymorphisms in *BCL2* to LTBI has not been previously studied. Although we found no associations of LTBI or TB with the *BCL2* variants rs1564483, rs956572, and rs12454712, it has been suggested that these may be associated with cancer[28] and the aging process[15], and therefore these polymorphisms deserve to be investigated further.

Studies in various countries and settings have shown that the rates of TB are significantly higher in males than in females[29], reflected by a male-to-female ratio for worldwide case notifications of 1.6. Host genetic factors may influence the overall outcome of *Mtb* infection and account for part of this disparity. Wu *et al.*[17] showed that the association of *STAT4* rs4853542 with TB was different in males than in females: the rs4853542*A allele may be more important in the development of male TB than female TB. Similarly, we found that *BCL2* rs80030866*G and rs9955190*G were associated with a decreased risk of TB in male subjects but not in female subjects. However, neither *BCL2* SNP was significantly associated with TB in males after the Bonferroni correction.

The standard duration of therapy for drug-sensitive pulmonary tuberculosis is 6 months[30], but treatment duration depends on the extent of the tuberculosis disease and the response to therapy. Poltavskaya *et al.*[31] showed that the rs8341*TT genotype appears to have a protective effect against a more severe form of schizophrenia. Similarly, our result showed that rs80030866*GA+GG was less common in patients who received treatment courses longer than the usual 6 months. However, this association is difficult to explain, as we included only newly diagnosed patients with drug-susceptible TB and found no significant association of *BCL2* variants with indications of TB disease severity such as sputum smear status or the presence of cavities. Saranathan *et al.*[32] also suggested that there was no significant association between *TIRAP* (Toll/interleukin-1 receptor domain-containing adaptor protein) variants and smear grade or cavity status among TB patients. The haplotype analysis found that the

rs80030866-rs9955190 haplotype was also associated with TB, with the AA haplotype again more frequent and the GG haplotype less frequent in the TB group when compared to the LTBI group.

The main weaknesses of our study were the limited sample size and the lack of a replication cohort to verify the associations that we have found. We recognize that this is a preliminary study and that further work is required to validate the associations we have identified and explore the underlying mechanisms.

CONCLUSION

In summary, our study suggests that *BCL2* gene polymorphisms may be correlated with the susceptibility to LTBI and TB in the Chinese Han population, which could potentially help to elucidate the relationship of apoptosis with the development of TB. To the best of our knowledge, this is the first report that associates *BCL2* polymorphisms with TB/LTBI susceptibility. These data suggest that the *BCL2* SNP associations that we have identified could perhaps serve as biomarkers for discriminating between latent and active TB infection.

AUTHORS' CONTRIBUTIONS

JH and SL participated in the design and the statistical analysis of the study and drafted the manuscript; YZ participated in the design of the study and revised the manuscript carefully; HT assisted in the English language editing; XG and FZ collected the data. All authors read and approved the final manuscript.

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