Infection, Genetics and Evolution 75 (2019) 103970

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Research paper

Association of *LEPR* polymorphisms with predisposition and inflammatory response in anti-tuberculosis drug-induced liver injury: A pilot prospective investigation in Western Chinese Han population

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ARTICLE INFO

Keywords:
Anti-tuberculosis drug-induced liver injury (ATLI)
Leptin receptor (*LEPR*)
Polymorphisms
Predisposition
Inflammatory response

ABSTRACT

Objectives: Previous studies have proposed leptin/leptin receptor (LEPR) pathway has a potential role in the oxidative stress induction as well as in immune and inflammatory responses; however, the effects of leptin/LEPR signaling on anti-tuberculosis drug-induced liver injury (ATLI) remain unexplored. Here, we aimed to investigate the potential relationships between *LEPR* polymorphisms and ATLI risk and clinical characteristics.

Methods: In total, this prospective study included 745 tuberculosis subjects with isoniazid and rifampin co-administration from West China. Six candidate single nucleotide polymorphisms (SNPs) in *LEPR* gene were genotyped by using a custom-by-design 48-Plex SNPscan kit. All subjects were monitored for six months to assess the occurrence of ATLI. Genetic association analysis at both the single-SNP and haplotype levels was performed. Significant SNPs were further explored in relation to clinical features and inflammatory response of ATLI cases.

Results: ATLI was identified in 118 of 745 subjects with a prevalence rate of 15.84%. Significant differences were observed in the allele and genotype distribution of *LEPR* rs2025804 in ATLI cases compared to non-ATLI controls (allele: OR = 1.64, 95% CI = 1.15–2.32, adjusted-p = .036; dominant model: OR = 1.73, 95% CI = 1.14–2.61, adjusted-p = 0.054; additive model: OR = 1.64, 95% CI = 1.15–2.34, adjusted-p = 0.036). Haplotype AA comprising of rs2025804 and rs2104564 was associated with a 1.58-fold increased predisposition to ATLI with p = 0.013. Furthermore, among ATLI patients, individuals carrying minor allele-containing genotypes in rs10889551, rs2025804 and rs2104564 loci had higher levels of C-reactive protein as compared to those homozygous major allele carriers, at p of 0.002, 0.057 and 0.012, respectively.

Conclusion: Ours is the first study which shows that *LEPR* polymorphisms may increase the risk for ATLI and may influence the inflammatory response in ATLI patients among Western Chinese Han tuberculosis patients.

1. Introduction

Tuberculosis (TB) is a major infectious disease that is associated with high morbidity and mortality across the world. In the Global Tuberculosis Report 2018, there were estimated 10.10 million new cases of TB worldwide in 2017 (World Health Organization, 2018). China accounts for about 9% of global TB cases and is ranked second among 30 countries with the highest TB burden (World Health Organization, 2018). Isoniazid (INH) and rifampin (RIF) are the most important first-line anti-tuberculosis (anti-TB) agents for controlling TB pandemics. Despite the therapeutic benefits of INH and RIF combination regimen, the use of this regimen is often associated with anti-TB drug-induced liver injury (ATLI). The rates of occurrence for ATLI range between 3%–28% issued by previous studies (Tweed et al., 2018; Tostmann et al., 2008). This condition could lead to non-compliance to treatment, poor treatment outcomes, emergence of drug-resistant *Mycobacterium tuberculosis* (MTB) strains, and even mortality (Tostmann et al., 2008; Munro et al., 2007). It is now considered that these adverse consequences of ATLI have posed a significant challenge to TB management in clinical practice.

Previous studies suggest that obesity may be one of the risk factors for drug-induced hepatotoxicity (Bessone et al., 2018). Adipokine leptin plays a central role in the regulation of body weight and is predominantly produced by adipose cells. Leptin binding to leptin receptor (LEPR) triggers the activation of downstream signaling molecules, and is able to inhibit food intake and enhance energy expenditure through

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https://doi.org/10.1016/j.meegid.2019.103970
Received 22 May 2019; Received in revised form 8 July 2019; Accepted 16 July 2019
Available online 17 July 2019
1567-1348/

its effects on the central nervous system (Crujeiras et al., 2015). The broad expression of LEPR in a variety of peripheral tissues could account for the leptin's pleiotropic functions on lipid metabolism, pancreatic cell function, hematopoiesis, thermogenesis, and response to lipopolysaccharide (Margetic et al., 2002). Currently, accumulative evidence has indicated that leptin plays a role in the modulation of cellular redox balance. For example, there are several reports regarding leptin-stimulated reactive oxygen species formation by various cell types, including keratinocytes (Savini et al., 2003), macrophages (Maingrette and Renier, 2003), endothelial cells (Bouloumie et al., 1999), cardiomyocytes (Xu et al., 2004) and hepatic stellate cells (De Minicis et al., 2008). In addition, the roles of leptin signal in modulating immunity and inflammation responses have become increasingly evident in the recent years (Procaccini et al., 2010). Some researchers have paid substantial attention to the involvement of the leptin signal in the pathophysiological mechanisms of immune-mediated diseases and inflammatory processes (Abella et al., 2017; Behnes et al., 2012; Sitaraman et al., 2004). Recent review by Abella et al. shows that there are intricate interactions between the leptin signaling and the immune system involving both innate and adaptive immunity (Abella et al., 2017). Behnes M et al. have observed the alterations in leptin generation during the course of acute infection and inflammation (Behnes et al., 2012). By *in vitro* and *in vivo* experiments, Sitaraman S et al. have demonstrated that the colonic-secreted leptin, as a novel pro-inflammatory cytokine, has potent effects on inflammatory bowel disease development and progression (Sitaraman et al., 2004).

At present, the pathogenesis of ATLI is believed to be drug-induced accumulation of toxic metabolite in the liver (Tostmann et al., 2008). These reactive metabolites are contributed to the production of the overbearing reactive oxygen species, resulting in oxidative stress and cell death (Ramappa and Aithal, 2013). Hepatic cellular dysfunction and death from ATLI can subsequently activate the immune and inflammatory responses (Chen et al., 2015). Many pro- and anti-inflammatory cytokine mediators are secreted during the immune and inflammatory responses. These cytokines are in a delicate balance, which determine progress to severe lesion or recovery (Ramappa and Aithal, 2013; Holt and Ju, 2006). Based on the available findings on the role of leptin/LEPR signaling in reactive oxygen species induction as well as in immune and inflammatory responses, we postulate that leptin/LEPR signal may participate in the development and progression of ATLI. Furthermore, studies document that leptin signal plays an important modulatory role in T cell-mediated liver toxicity. Using two distinct mice models, Faggioni and colleagues have observed the diminished susceptibility of leptin-deficient mice to Concanavalin A- and *Pseudomonas aeruginosa* exotoxin A-induced hepatotoxicity, which may ascribe to the defective production of tumor necrosis factor (TNF) α and interleukin (IL) 18 in leptin-deficient mice (Faggioni et al., 2000). However, very few studies illustrating the potential link between leptin/LEPR signaling and ATLI have been carried out.

Throughout the years, the influence on ATLI by genetic risk factors has been extensively investigated. The genetic variants associated with ATLI have been identified in some protein-encoding genes, such as *N-acetylransferase 2*, *pregnane X receptor*, *human leukocyte antigens*, and *tumor necrosis factor-α* (Bao et al., 2018). To assess the possible relationship between leptin/LEPR pathway and ATLI occurrence, we selected *LEPR* gene as putative ATLI-susceptibility gene and conducted a candidate gene-based association study. Here, we analyzed the associations between six single nucleotide polymorphisms (SNPs) within *LEPR* gene and occurrence risk for ATLI and its clinical phenotypes in Western Chinese Han patients with TB. The present study aims to provide useful information in understanding the potential effect of leptin/LEPR signaling on ATLI occurrence and progression. Furthermore, the positive results could be used to identify TB patients at risk of developing ATLI, and may help to reduce the potential risk and incidences of hepatic injury.

2. Materials and methods

2.1. Study population

In total, 1060 patients suspected with TB were consecutively recruited in this prospective trial at West China Hospital of Sichuan University, Western China, between Dec. 2016 and Apr. 2018. Subsequently, 817 patients were diagnosed with active TB disease by experienced physicians based on typical TB clinical symptoms, microbiological and molecular etiological evidence for MTB, radiographic results, and appropriate response to anti-TB treatment. All patients enrolled received therapy that at least included INH (300–400 mg daily) and RIF (450–600 mg daily) for six months or more. Sixteen patients with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) infections were excluded. Twenty-eight subjects who presented with abnormal liver biochemistry tests prior to anti-TB chemotherapy were also excluded. Thirteen cases were lost due to poor compliance to treatment or withdrawal from the 6-month follow-up. Four cases with TB received other hepatotoxic drugs, while ten cases changed medicine regimen that included second-line drugs during anti-TB treatment. A DNA sample for one individual failed to genotype for all targeted SNPs. Overall, 745 patients with active TB were finally enrolled in this prospective study.

The demographic and clinical data of patients were obtained from the electronic medical records. Blood samples were obtained from each patient for genotyping assay. Before initiating anti-TB treatment, biochemical test and peripheral blood cell counts were carried out. After treatment, measurements of liver function indicators were executed twice a month during the first two months and monthly in the remaining four months, which included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). All subjects were monitored for six months. The automatic hematology analyzer SYSMEX XN-10 (Sysmex, Japan) was used to perform the peripheral blood cell count examination; the biochemical test was carried out by using Cobas c702 analyzer (Roche, Germany); erythrocyte sediment rate (ESR) was determined in automatic ESR analyzer test (ALIFAX, Italy); C-reactive protein (CRP) level was measured by employing the automated analyzer IMMAGE 800 (Beckman Coulter). All aforementioned laboratory tests were performed according to the manufacturers' protocol and were carried out in the West China Hospital which is certified by the College of American Pathologists (CAP).

Fig. 1 shows the study protocol used to enroll patients. All participants were unrelated Han Chinese and each participant provided a written informed consent. The protocol of the present study was approved by the Clinical Trials and Biomedical Ethics Committee of West China Hospital, Sichuan University (reference no. 198; 2014).

2.2. Definition, severity and clinical presentation of ATLI case

When ALT levels were > 3 times the upper limit of normal (ULN) (120 IU/L) together with hepatitis-related symptoms, or ALT levels ≥5 × the ULN (150 IU/L) with or without clinical manifestations, either situation was considered to be ATLI (Nahid et al., 2016). All TB patients with ATLI suspicion were reviewed by a panel of two physicians with ATLI expertise during the 6-month follow-up. According to classification criteria issued by the World Health Organization (WHO), the severity of ATLI was defined as mild, moderate, and severe: Grade 1 (mild), ALT < 2.5 ULN; Grade 2 (mild), ALT 2.5–5 ULN; Grade 3 (moderate), ALT 5–10 ULN; Grade 4 (severe), ALT > 10 ULN (Tostmann et al., 2008). The clinical presentations of ATLI were classified as hepatocellular (R value ≥5), cholestatic ($R \leq 2$) and mixed (R-value between 2 and 5) types (Leise et al., 2014). The R value was obtained based on the liver biochemistry test, calculated as R-value = serum [ALT/ALT ULN] / [ALP/ALP ULN].

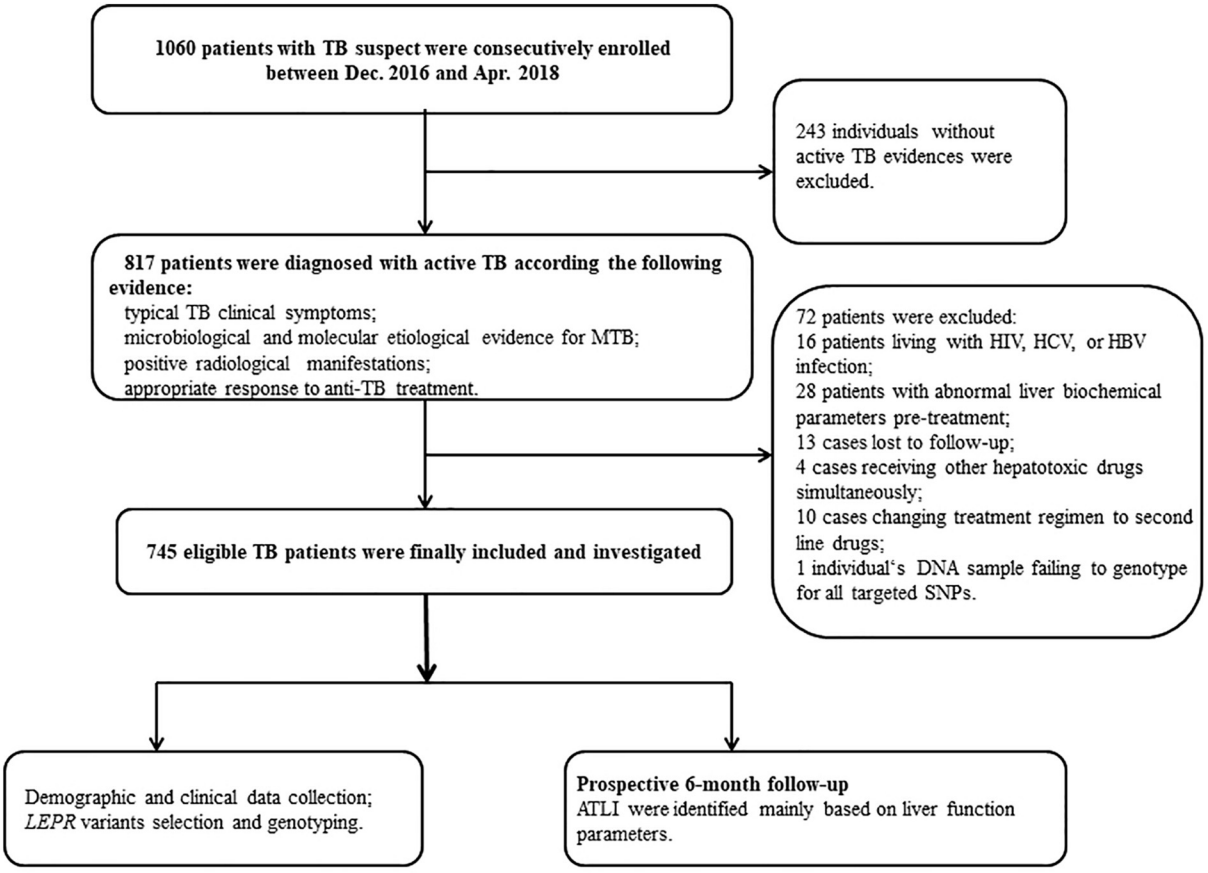

Fig. 1. A flow diagram for the protocol used to recruit participants.

2.3. Determination of candidate polymorphisms and genotyping

LEPR gene is located on chromosome 1q31.3 and encodes the specific receptor for anti-obesity hormone leptin. We selected SNP candidates within the highly polymorphic *LEPR* gene through the following steps: (1) the genetic polymorphism data of the full sequence of *LEPR* was obtained by performing thorough searches on dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/); (2) all SNPs were filtered based on the minor allele frequency (MAF) of 0.05 or more among Han Chinese in Beijing, China (CHB); (3) SNP loci were preferentially included if they were located in the promoter, exon, untranslated region (UTR), intron and potential regulatory regions. Together with previous studies concerning *LEPR* genetic variations associated with other diseases, six SNPs within *LEPR* (rs10889551, rs2025804, rs2104564, rs7413467, rs1137101, and rs1805134) were eventually selected for analysis.

Genomic DNA was extracted from whole blood sample. SNP genotyping was determined by using a custom-by-design 48-Plex SNPscan kit (Genesky Biotechnologies Inc., Shanghai, China). This commercial kit was developed based on patented SNP genotyping technology, with double ligation and multiplex fluorescence polymerase chain reaction. The genotyping assay was performed according to the detailed method reported previously (Hu et al., 2018; Zhang et al., 2018). To ensure genotyping quality, repeated genotyping experiments were performed in approximately 10% specimens randomly selected from all DNA samples which a concordance rate of 100% was obtained. Moreover, five samples containing different genotypes for each SNP were chosen to verify the genotyping results by applying direct sequencing method.

2.4. Statistical analysis

Data were expressed as the mean ± standard deviation (SD), median (interquartile range, IQR) or number (frequency), depending on the data characteristics. Demographic variables and baseline laboratory results were compared between patient group with and without ATLI using univariate analysis, in which categorical variables were tested with the Chi-square test, whereas continuous variables were examined using the Student *t*-test or Mann-Whitney *U* test. Furthermore, we tested for whether *LEPR* polymorphisms were associated with clinical features of ATLI patients. The above-mentioned analyses were performed using SPSS V22.0 software (IBM, Chicago, IL, USA).

Hardy-Weinberg equilibrium (HWE) for all SNPs in controls without ATLI was assessed using the goodness-of-fit Chi-square test. Associations between *LEPR* SNPs and risk of ATLI were evaluated based on the allele and genotypic frequencies, as well as the dominant, recessive and additive genetic model distributions. Subsequently, we examined the potential correlations between *LEPR* variants and hepatotoxicity predisposition in the subsets of ATLI patients who manifested with distinct severity and clinical pattern. Odd ratio (OR) and 95% confidence interval (CI) were calculated. The above-mentioned analysis was carried out by PLINK v1.07 software. Bonferroni corrections were employed to correct for multiple tests. Furthermore, we employed Haploview v4.2 software to establish the linkage disequilibrium block and to build haplotypes among the SNPs selected. The Generalized Multifactor Dimensionality Reduction (GMDR) v0.9 software was adopted to identify potential interaction models among 6 *LEPR* polymorphic loci that associated with ATLI risk. The best interaction models for each locus were screened under the highest cross-validation consistency (CVC) and the highest testing balance accuracy (TBA); moreover the statistical *p*-value was calculated. All statistical tests were two-

Table 1
The clinical features of TB patients with and without ATLI.

Clinical features	All patients	TB with ATLI	TB without ATLI	*P*
	N = 745	*N* = 118	*N* = 627	
Gender, male/female	444/301(59.60%)	69/49(58.47%)	375/252(59.81%)	0.786
Age, mean ± SD (years)	42.53 ± 18.04	40.92 ± 15.72	42.83 ± 18.44	0.290
Weight, mean ± SD (kg)	54.30 ± 10.10	55.04 ± 9.06	54.15 ± 10.30	0.440
Smoking, Yes/No	227/518(30.47%)	35/83(29.66%)	192/435(30.62%)	0.835
Drinking, Yes/No	172/573(23.09%)	32/86(27.12%)	140/487(22.33%)	0.257
TB subtype, n (%)				0.069
PTB	522(70.7)	78(66.10)	444(70.81)	
EPTB	71(9.53)	18(15.25)	53(8.45)	
PTB & EPTB	152(20.40)	22(18.64)	130(20.74)	
General Symptoms, n (%)	518(69.53)	89(75.42)	429(68.42)	0.129
Fever	309(41.48)	63(53.39)	246(39.23)	**0.004**
Weight loss	259(34.77)	34(28.81)	225(35.89)	0.139
Night sweat	195(26.17)	29(24.58)	166(26.48)	0.667
Poor appetite	263(35.30)	45(38.14)	218(34.71)	0.483
Fatigue	173(23.22)	31(26.27)	142(22.65)	0.392
Local infectious symptoms, n (%)	519(69.66)	88(74.58)	431(68.74)	0.206
Laboratory indicators-baseline				
TBIL, μmol/L	8.90(6.60–12.60)	10.15(7.5–14.53)	8.80(6.40–12.20)	**0.002**
DBIL, μmol/L	3.50(2.50–5.50)	3.70(2.50–6.73)	3.50(2.50–5.40)	0.127
ALT, IU/L	15.00(10.00–24.00)	27.00(15.00–38.00)	15.00(10.00–21.00)	< **0.001**
AST, IU/L	21.00(16.00–27.00)	26.50(19.75–34.00)	20.00(16.00–25.00)	< **0.001**
ALP, IU/L	81.00(64.00–98.50)	84.00(67.00–105.00)	79.00(64.00–97.00)	**0.020**
GGT, IU/L	31.00(19.00–52.00)	43.00(26.00–78.00)	29.00(18.00–48.00)	< **0.001**
Laboratory indicators-peak				
TBIL, μmol/L		12.10(8.50–18.32)		
DBIL, μmol/L		5.45(3.62–9.88)		
ALT, IU/L		132.50(57.50–206.75)		
AST, IU/L		95.50(41.00–173.00)		
ALP, IU/L		102.50(73.00–171.50)		
GGT, IU/L		77.50(43.25–151.00)		

Notes: TB: tuberculosis, ATLI: antituberculosis drug-induced liver injury, PTB: pulmonary tuberculosis, EPTB: extrapulmonary tuberculosis, PTB & EPTB: pulmonary tuberculosis along with extra-pulmonary tuberculosis, TBIL: total bilirubin, DBIL: direct bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase.
Bold indicates $p < 0.05$

sided and $p < 0.05$ was considered statistical significant.

3. Results

3.1. Patient characteristics

The demographic and clinical details of TB patient are provided in Table 1. In total, 118 TB patients were diagnosed with cases of ATLI among the 745 study subjects who received INH and RIF's cotreatment. The prevalence rate of ATLI was 15.84% (118/745). Two patients out of 118 ATLI cases did not provided the detailed results of liver functions examination for further analysis. Our ATLI cohort consisted of 82 mild, 20 moderate and 14 severe hepatotoxicity subjects. With regard to clinical presentation of ATLI, hepatocellular liver injury accounted for approximately half of all ATLI cases (63, 54.31%), the following were cholestatic (30, 25.86%) and mixed class (23, 19.83%).

None significant differences were observed in gender and age between patients with and without ATLI ($p = 0.786$ and $p = 0.290$, respectively). Both groups predominantly consisted of middle-aged males, and males accounted for 59.60% of all patients. The average age of 745 patients was 42.53 years of age. Among patients having ATLI, smokers and drinkers were 29.66% and 27.12%, respectively, while the proportion of smokers and drinkers in patients without ATLI were 30.62% and 22.33%, respectively ($p = 0.835$, $p = 0.257$). The body weight was similar between ATLI group and non-ATLI group (55.04 ± 9.06 kg *vs.* 54.15 ± 10.30 kg, $p = 0.440$). Most patients (69.66%) displayed local infectious symptoms, such as cough and sputum upon admission. We also observed a cluster of TB-associated clinical presentations including fever, weight loss, night sweat, poor appetite, and fatigue. Among these clinical manifestations, there was a significant difference between ATLI and non-ATLI individuals in the proportion of fever occurrence (53.39% *vs.* 39.23%, $p = 0.004$). In the study cohort, there were 522 pulmonary tuberculosis (PTB) patients, 71 extrapulmonary tuberculosis (EPTB) patients, and 152 PTB along with EPTB cases (PTB & EPTB). The distribution of TB subtypes did not statistically differ between patients with and without ATLI ($p = 0.069$).

Quantitative laboratory results of the participants at baseline and at peak during anti-TB chemotherapy are shown in Table 1. Before anti-TB treatment, liver function markers that such as, TBIL, ALT, AST, ALP, and GGT were significantly higher levels in ATLI patients relative to those without ATLI ($p = 0.002$ for TBIL, $p < 0.001$ for ALT, $p < 0.001$ for AST, $p = 0.020$ for ALP, and $p < 0.001$ for GGT). But, all these markers were within normal range in either ATLI group or non-ATLI group. As expected, liver function indexes increased significantly during the 6-month follow-up in patients bearing ATLI ($p < 0.001$ for DBIL, $p = 0.019$ for TBIL, $p < 0.001$ for ALT, $p < 0.001$ for AST, $p = 0.001$ for ALP, and $p < 0.001$ for GGT, Supplementary Fig. S1).

3.2. Genotyping results

Genotyping was not successful in four patients at four SNP loci, rs10889551, rs2104564, rs1137101, and rs1805134. Genotyping of SNPs rs2025804 and rs7413467 was successfully completed for 118 ATLI patients and 627 controls without hepatotoxicity. The information regarding the genotyped SNPs is described in detail in Supplementary Table S1, in terms of chromosomal locations, molecular consequences, minor allele frequencies as well as *p*-values for Hardy-Weinberg equilibrium (HWE) test. Genotype distribution of all six SNPs within *LEPR* gene in the 627 participants free of ATLI conformed to HWE ($p > .05$ for all loci). The minor allele frequencies of six *LEPR* variants found

Table 2
Association between *LEPR* genetic variants and ATLI risk in Han Chinese population.

SNP	Allele	Case n(%)	Control n(%)	OR (95% CI)	*P*	*P***	Variant	Case n(%)	Control n(%)	*P*	*P***
rs10889551	G	55(23.31)	214(17.09)	1.47(1.05–2.06)	**0.023**	0.138	GG	5(4.24)	20(3.19)	0.054	
	A	181(76.69)	1038(82.91)	1	–		GA	45(38.13)	174(27.80)		
							AA	68(57.63)	432(69.01)		
rs2025804	A	50(21.19)	177(14.11)	1.64(1.15–2.32)	**0.006**	**0.036**	AA	5(4.24)	12(1.91)	**0.021**	
	G	186(78.81)	1077(85.89)	1	–		AG	40(33.90)	153(24.20)		0.126
							GG	73(61.86)	462(73.69)		
rs2104564	A	46(19.49)	170(13.58)	1.54(1.08–2.21)	**0.018**	0.108	AA	5(4.24)	12(1.92)	0.059	
	G	190(80.51)	1082(86.42)	1	–		AG	36(30.51)	146(23.32)		
							GG	77(65.25)	468(74.76)		
rs7413467	A	49(20.76)	199(15.87)	1.39(0.98–1.97)	0.064	0.384	AA	5(4.24)	15(2.39)	0.175	
	G	187(79.24)	1055(84.13)	1	–		AG	39(33.05)	169(26.95)		
							GG	74(62.71)	443(70.66)		
rs1137101	A	34(14.41)	147(11.74)	1.27(0.85–1.89)	0.251		AA	4(3.39)	14(2.24)	0.540	
	G	202(85.59)	1105(88.26)	1	–		AG	26(22.03)	119(19.01)		
							GG	88(74.58)	493(78.75)		
rs1805134	G	20(8.47)	86(6.87)	1.26(0.76–2.09)	0.379		GG	2(1.69)	4(0.64)	0.467	
	A	216(91.53)	1166(93.13)	1	–		GA	16(13.56)	78(12.46)		
							AA	100(84.75)	544(86.90)		

Notes: *P*: *p* value was calculated by Chi-square test.
*P***: *p* value after Bonferroni correction.

here were in accord with those in the CHB population reported by the 1000 Genome Project (phase 3).

3.3. Association of genetic variants of LEPR gene with ATLI predisposition

The allele and genotype distributions of six SNPs in *LEPR* gene in ATLI cases and non-ATLI controls are summarized in Table 2. In *LEPR* gene, the allelic frequencies of rs10889551, rs2025804 and rs2104564 markedly differed between cases and controls. Subjects with minor alleles (G and A) of the two SNPs rs10889551 and rs2104564 were associated with an increase in susceptibility to ATLI occurrence, with OR of 1.47–1.54, separately (p = .023 for rs10889551 and p = .018 for rs2104564). The differences of these two SNPs between TB patients with and without ATLI disappeared after adjusting with Bonferroni method. Another intron variant, rs2025804, was strongly associated with the increased risk for ATLI. Its A allele frequency was 21.19% in the case group and 14.11% in control group, and p value was 0.036 after correction with Bonferroni algorithm (OR = 1.64, 95% CI = 1.15–2.32). No other significant differences in allele and genotype frequencies of these six SNPs were observed between patients with hepatotoxicity and the controls.

Subsequently, we performed dominant, recessive and additive genetic model analysis to explore differences in polymorphism distributions between cases and controls. The results of the analysis are displayed in Table 3. Of note, the dominant and additive models of rs2025804 were detected to be significantly associated with risk of developing ATLI. TB patients with rs2025804 A allele-involving genotype (AA and AG) exhibited a greater risk (1.73-fold) for ATLI when compared with those carrying homozygous GG genotype, with 95% CI = 1.14–2.61 (p = 0.009, and p = 0.054 after Bonferroni correction). Further, in the additive genetic pattern, rs2025804 minor allele A was closely related to ATLI susceptibility (OR = 1.64, 95% CI = 1.15–2.34, p = 0.006, Bonferroni-adjusted p = 0.036). Additionally, we observed that rs10889551 and rs2104564 potentially correlated with the elevated risk for ATLI occurrence under the dominant and additive model (rs10889551: GG + GA *vs.* AA p = 0.016, GG *vs.* AA p = 0.024; rs2104564: AA + AG *vs.* GG p = 0.033, AA *vs* GG p = 0.020). However, these discrepancies did not reach statistical significance after multiple comparison correction.

3.4. Subgroup analysis

We conducted the extended analysis in subsets of patients who were classified by severity and clinical presentation of ATLI. Owning to the limited number of ATLI cases, we only performed the dominant model analysis in these subgroup comparisons. The detailed results are presented in Table 4. SNP rs10889551 was associated with the mild and severe hepatotoxicity risk under the dominant model analysis (OR = 1.66, 95% CI = 1.04–2.65, p = 0.034; OR = 2.79, 95% CI = 1.02–8.67, p = 0.047, respectively). The *LEPR* gene rs2025804 A allele was found to correlate with an elevated susceptibility to ATLI in the mild hepatotoxicity subgroup (OR = 1.79, 95% CI = 1.11–2.89, p = 0.017). Under the dominant pattern, we also observed correlations between rs2025804 and risk of hepatocellular and cholestatic liver damage subgroups (OR = 1.72, 95% CI = 1.00–2.95, p = 0.048; OR = 2.14, 95% CI = 1.02–4.50, p = 0.045, respectively). Unfortunately, all these p-values were not statistical significance after multiple comparison correction (adjusted $p > 0.05$).

Table 3
Comparison of *LEPR* gene polymorphisms in relation to ATLI risk in Han Chinese population.

SNP	Dominant genetic model			Recessive genetic model			Additive genetic model		
	OR (95% CI)	*P**	*P***	OR (95% CI)	*P**	*P***	OR (95% CI)	*P**	*P***
rs10889551	1.64(1.10–2.45)	**0.016**	0.096	1.34(0.49–3.65)	0.566		1.47(1.05–2.06)	**0.024**	0.144
rs2025804	1.73(1.14–2.61)	**0.009**	**0.054**	2.27(0.78–6.56)	0.131		1.64(1.15–2.34)	**0.006**	**0.036**
rs2104564	1.58(1.04–2.40)	**0.033**	0.198	2.26(0.78–6.55)	0.132		1.53(1.07–2.20)	**0.020**	0.120
rs7413467	1.43(0.95–2.16)	0.087		1.81(0.64–5.07)	0.262		1.39(0.98–1.98)	0.065	
rs1137101	1.26(0.80–2.00)	0.315		1.53(0.50–4.74)	0.458		1.24(0.84–1.82)	0.272	
rs1805134	1.19(0.69–2.08)	0.529		2.68(0.49–14.81)	0.258		1.24(0.76–2.04)	0.391	

Notes: *P* *: *p* value was calculated using univariate logistic regression analysis.
*P***: *p* value after Bonferroni correction.

Table 4
Correlation of *LEPR* genetic variants with ATLI risk in subgroup analysis.

SNP	Mild (Grade 1 and Grade 2)			Moderate (Grade 3)			Severe (Grade 4)		
	OR (95% CI)	*P**	*P***	OR (95% CI)	*P**	*P***	OR (95% CI)	*P**	*P***
rs10889551	1.66(1.04–2.65)	**0.034**	0.204	0.95(0.36–2.52)	0.925		2.97(1.02–8.67)	**0.047**	0.282
rs2025804	1.79(1.11–2.89)	**0.017**	0.102	1.51(0.59–3.84)	0.390		1.56(0.51–4.71)	0.434	
rs2104564	1.62(1.00–2.64)	0.052		1.27(0.48–3.36)	0.631		1.65(0.54–4.98)	0.378	
rs7413467	1.62(1.01–2.60)	0.046		1.03(0.39–2.73)	0.950		0.96(0.30–3.11)	0.950	
rs1137101	1.20(0.70–2.05)	0.516		1.24(0.44–3.46)	0.687		1.48(0.46–4.80)	0.511	
rs1805134	1.36(0.73–2.54)	0.325		0.74(0.168–3.24)	0.686		1.11(0.24–5.03)	0.897	

SNP	Hepatocellular type (R ≧ 5)			Cholestatic type (R ≦ 2)			Mixed type (R 2–5)		
	OR (95% CI)	*P**	*P***	OR (95% CI)	*P**	*P***	OR (95% CI)	*P**	*P***
rs10889551	1.36(0.80–2.34)	0.249		1.95(0.93–4.07)	0.076		2.04(0.89–4.71)	0.094	
rs2025804	1.72(1.00–2.95)	**0.048**	0.288	2.14(1.02–4.50)	**0.045**	0.270	1.23(0.50–3.03)	0.661	
rs2104564	1.59(0.92–2.75)	0.100		1.98(0.93–4.19)	0.076		1.05(0.41–2.70)	0.927	
rs7413467	1.48(0.87–2.53)	0.151		1.61(0.76–3.40)	0.217		1.05(0.43–2.60)	0.910	
rs1137101	1.48(0.83–2.65)	0.183		1.13(0.47–2.69)	0.785		0.78(0.26–2.33)	0.657	
rs1805134	1.40(0.70–2.80)	0.336		1.66(0.66–4.18)	0.283		0.30(0.04–2.27)	0.244	

Notes: *P* *: *p* value was calculated using univariate logistic regression analysis.
*P***: *p* value after Bonferroni correction.

3.5. Haplotype construction

The linkage disequilibrium (LD) analysis and haplotype construction were also carried out. Fig. 2 shows that two *LEPR* variants (rs2025804 and rs2104564) were in high linkage disequilibrium (LD) with each other under a threshold of pairwise $r^2 \geq 0.80$. Haplotype blocks were built between rs2025804 and rs2104564, and two haplotypes were identified, that is, GG and AA. Haplotype frequencies and associations with ATLI risk are shown in Table 5. Compared with haplotype GG frequencies, the uncommon haplotype AA was associated with a significant raise in the risk for ATLI occurrence during anti-TB treatment, with $p = 0.013$ at OR of 1.58–95% CI ranging 1.10–2.27.

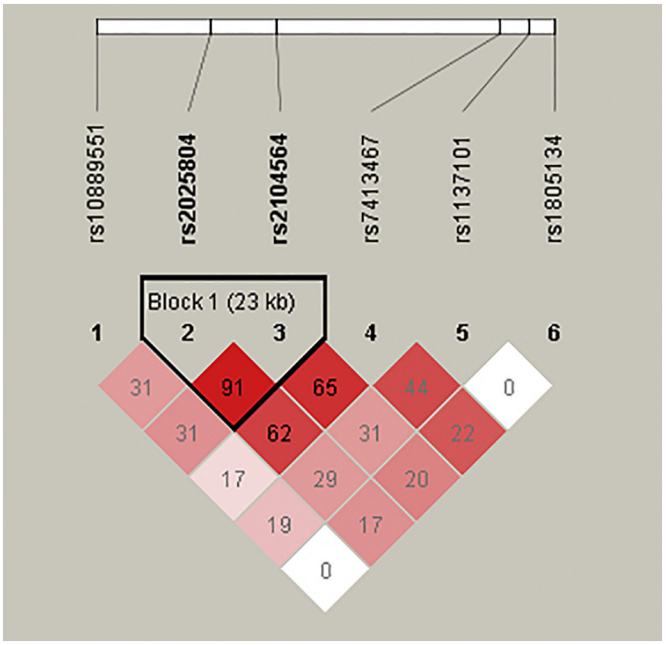

Fig. 2. The linkage disequilibrium (LD) pattern and haplotype block. Variants rs2025804 and rs2104564 were in strong LD and constructed two haplotypes, GG and AA.

Table 5
Haplotype constructions of the *LEPR* variants associated with the risk of ATLI.

Haplotype	Frequency				
	All	Cases with ATLI	Controls without ATLI	OR (95%CI)	*P*
GG	0.845	0.788	0.856	1.00	–
AA	0.144	0.195	0.134	**1.58 (1.10–2.27)**	0.013

Notes: *P: p* value was calculated using Chi-square test.

3.6. The interaction analysis

Obtained by GMDR method, the best model for predicting ATLI risk was the six-factor pattern that composed of rs10889551, rs2025804, rs2104564, rs7413467, rs1137101 and rs1805134, with CVC = 10/10 and TBA = 0.5285 (listed in Table 6). However, no statistically significant interactions among all analyzed SNPs were determined ($p > 0.05$), suggesting that there was no combined effect of six SNPs of *LEPR* gene on ATLI predisposition.

3.7. Clinical significance of LEPR SNPs in ATLI patients

For heavily exploring the potential clinical significance of *LEPR*

Table 6
Interplay models of *LEPR* genetic variants adopting the GMDR method.

Model	Training balance accuracy	Testing balance accuracy	Cross-validation consistency	Sign *p*-value
A2	0.5616	0.5188	6/10	0.828
A1 A2	0.5707	0.5316	5/10	0.377
A1 A2 A6	0.5849	0.5397	8/10	0.377
A1 A2 A4 A5	0.5912	0.5147	4/10	0.377
A1 A2 A3 A4 A5	0.5983	0.5229	6/10	0.623
A1 A2 A3 A4 A5 A6	0.6039	0.5285	10/10	0.172

Notes: A1: rs10889551, A2: rs2025804, A3: rs2104564, A4: rs7413467, A5: rs1137101, A6: rs1805134.
Sign *p*-value was calculated by GMDR v0.9 software.

Table 7
Association of SNP rs10889551 with clinical traits of patients with ATLI.

	rs10889551		
Variables	GG + GA(N = 50)	AA(N = 68)	P-value
Basic information			
Gender, male/female	27/23(54.00%)	442/26(61.76%)	0.398
Age, mean ± SD (years)	42.02 ± 16.32	40.10 ± 15.32	0.515
Weight, mean ± SD (kg)	52.58 ± 8,73	56.69 ± 8.98	**0.032**
Clinical phenotype, n(%)			
General Symptoms	40(80.00)	49(72.06)	0.322
Fever	26(52.00)	37(54.41)	0.795
Weight Loss	16(32.00)	18(26.47)	0.512
Night Sweat	13(26.00)	16(23.53)	0.758
Poor Appetite	22(44.00)	23(33.82)	0.261
Fatigue	16(32.00)	15(22.06)	0.225
Local infectious symptoms	35(70.00)	53(77.94)	0.328
Blood cell counts			
RBC, ×1012/L	4.11 ± 1.05	4.19 ± 0.92	0.646
PLT, ×109/L	217.94 ± 105.51	210.47 ± 101.76	0.708
Leucocytes, ×109/L	7.35 ± 4.28	5.90 ± 2.61	**0.030**
Neutrophils, ×109/L	5.32 ± 3.90	4.08 ± 2.34	**0.040**
Lymphocyte, ×109/L	1.28 ± 0.82	1.18 ± 0.55	0.459
Monocyte, ×109/L	0.43(0.29–0.63)	0.38(0.29–0.67)	0.814
System inflammatory indicators			
ESR, mm/h	47.00(23.00–67.50)	35.00(15.00–57.50)	**0.047**
CRP, mg/L	19.40(5.23–77.27)	6.53(1.98–22.90)	**0.002**
Liver function parameters, peak			
TBIL, μmol/L	12.00(7.70–19.10)	12.90(8.50–18.50)	0.526
DBIL, μmol/L	5.6(3.65–9.10)	5.30(3.50–10.40)	0.975
ALT, IU/L	107.00(56.00–221.50)	155.00(69.00–206.00)	0.368
AST, IU/L	85.00(38.50–197.00)	103.00(47.00–161.00)	0.973
ALP, IU/L	94.00(69.00–207.00)	106.00(73.00–166.00)	0.729
GGT, IU/L	63.00(38.50–150.00)	83.00(46.00–159.00)	0.454

Notes: RBC: red blood cell, PLT: platelet, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, TBIL: total bilirubin, DBIL: direct bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase.

Table 8
Association of significant rs2025804 with clinical traits of patients with ATLI.

	rs2025804		
Variables	AA + AG(N = 45)	GG(N = 73)	P-value
Basic information			
Gender, male/female	24/21(53.33%)	45/28(61.64%)	0.374
Age, mean ± SD (years)	42.64 ± 16.19	39.85 ± 15.43	0.350
Weight, mean ± SD (kg)	52.74 ± 7.27	56.32 ± 9.75	0.069
Clinical phenotype, n(%)			
General Symptoms	36(80.00)	53(72.60)	0.365
Fever	27(60.00)	36(49.32)	0.258
Weight Loss	14(31.11)	20(27.40)	0.665
Night Sweat	16(35.56)	13(17.81)	0.062
Poor Appetite	20(44.44)	25(34.25)	0.268
Fatigue	13(28.89)	18(24.66)	0.852
Local infectious symptoms	32(71.11)	56(76.71)	0.497
Blood cell counts			
RBC, ×1012/L	4.05 ± 1.10	4.22 ± 0.90	0.394
PLT, ×109/L	212.34 ± 101.97	214.55 ± 104.34	0.914
Leucocytes, ×109/L	6.44 ± 3.37	6.59 ± 3.60	0.827
Neutrophils, ×109/L	4.52 ± 2.91	4.68 ± 3.32	0.802
Lymphocyte, ×109/L	1.19 ± 0.80	1.24 ± 0.61	0.752
Monocyte, ×109/L	0.36(0.29–0.60)	0.45(0.29–0.71)	0.180
System inflammatory indicators			
ESR, mm/h	47.00(24.00–66.00)	35.00(16.00–60.50)	0.104
CRP, mg/L	20.90(4.66–55.20)	8.41(2.29–23.35)	**0.057**
Liver function parameters, peak			
TBIL, μmol/L	10.15(6.73–17.58)	13.05(9.43–19.55)	0.081
DBIL, μmol/L	5.15(3.08–8.50)	5.75(3.80–10.33)	0.263
ALT, IU/L	108.00(51.00–207.00)	140.50(60.50–206.75)	0.390
AST, IU/L	103.50(37.75–183.50)	89.50(44.75–162.50)	0.991
ALP, IU/L	97.50(68.25–197.75)	105.00(75.25–169.00)	0.635
GGT, IU/L	62.00(43.25–162.75)	83.50(43.00–144.75)	0.613

Notes: RBC: red blood cell, PLT: platelet, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, TBIL: total bilirubin, DBIL: direct bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase.

Table 9
Association of SNP rs2104564 with clinical traits of patients with ATLI.

	rs2104564		
Variables	AA + AG(N = 41)	GG(N = 77)	P-value
Basic information			
Gender, male/female	24(58.53%)	45(58.44%)	0.992
Age, mean ± SD (years)	42.41 ± 16.26	40.12 ± 15.46	0.452
Weight, mean ± SD (kg)	53.00 ± 7.31	55.98 ± 9.67	0.144
Clinical phenotype, n(%)			
General Symptoms	32(78.05)	57(74.03)	0.629
Fever	24(58.54)	39(50.65)	0.413
Weight Loss	13(31.71)	21(27.27)	0.613
Night Sweat	14(34.15)	15(19.48)	0.078
Poor Appetite	17(41.46)	28(36.36)	0.587
Fatigue	12(29.27)	19(24.67)	0.589
Local infectious symptoms	29(70.73)	59(76.62)	0.484
Blood cell counts			
RBC, ×1012/L	4.04 ± 1.04	4.21 ± 0.94	0.394
PLT, ×109/L	215.59 ± 106.45	212.78 ± 101.94	0.893
Leucocytes, ×109/L	6.61 ± 3.48	6.50 ± 3.53	0.880
Neutrophils, ×109/L	4.69 ± 2.97	4.59 ± 3.27	0.872
Lymphocyte, ×109/L	1.18 ± 0.82	1.24 ± 0.60	0.676
Monocyte, ×109/L	0.36(0.28–0.60)	0.44(0.29–0.68)	0.147
System inflammatory indicators			
ESR, mm/h	47.00(24.00–71.00)	35.00(17.50–60.50)	0.085
CRP, mg/L	22.00(4.90–59.70)	7.52(2.29–22.60)	**0.012**
Liver function parameters, peak			
TBIL, μmol/L	10.30(6.65–17.57)	12.90(8.90–19.55)	0.173
DBIL, μmol/L	5.30(3.07–8.65)	5.65(3.80–10.07)	0.363
ALT, IU/L	108.00(51.00–207.00)	139.50(59.25–206.75)	0.559
AST, IU/L	103.50(37.75–183.50)	89.50(44.75–162.50)	0.898
ALP, IU/L	96.00(68.25–202.00)	104.00(75.25–169.00)	0.734
GGT, IU/L	61.00(43.25–162.75)	84.50(43.00–144.75)	0.480

Notes: RBC: red blood cell, PLT: platelet, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, TBIL: total bilirubin, DBIL: direct bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase.

genetic polymorphisms, we investigated the probable associations between three *LEPR* SNPs (rs10889551, rs2025804 and rs2104564 that potentially associated with ATLI predisposition) and ATLI cases' clinical and laboratory characteristics. In ATLI patients, most indicators were comparable in both comparisons in rs10889551, rs2025804 and rs2104564 loci as shown in Table 7, Table 8 and Table 9, respectively. For rs10889551, significant differences were found in body weight as well as in peripheral levels of leucocyte numbers, neutrophils numbers, ESR, and CRP in GG + GA group compared to AA group (p = 0.032, 0.030, 0.040, 0.047 and 0.002, respectively). It is noteworthy that, in rs10889551 locus, ATLI individuals with GG/GA genotype had a median level of CRP of 19.40 mg/L (IQR = 5.23–77.27 mg/L), whereas ATLI cases with AA genotype had a median level of 6.53 mg/L (IQR = 1.98–22.90 mg/L) of CRP (Table 7). Moreover, genetic variations in rs2025804 seemed to be correlated with peripheral CRP levels, in which ATLI subjects carrying A allele-containing genotypes were more likely to have higher levels of peripheral CRP (p-value of 0.057, Table 8). Furthermore, in the case of SNP rs2104564, a significant difference was seen with regard to serum CRP levels in the dominant model analyses (p = 0.012). The levels of serum CRP were significantly higher when AA + AG group compared to GG group (22.00 (IQR = 4.90–59.70) mg/L *vs.* 7.52 (IQR = 2.29–22.60) mg/L, Table 9). As presented in Fig. 3, SNPs rs10889551, rs2025804 and rs2104564 might be associated with CRP expression levels in individuals with ATLI; however, there was no combined effect of these three *LEPR* SNPs on CRP levels (p > 0.05, data not shown).

4. Discussion

ATLI is associated with significant morbidity and mortality which substantially reduces the effectiveness of anti-TB treatment. Though there is no paper regarding association of *LEPR* with ATLI, there are reports showing association of *LEPR* polymorphism with non-alcoholic liver disease (Swellam and Hamdy, 2012; Zain et al., 2013). Moreover, previous studies have proposed that leptin/ LEPR pathway has a potential role in oxidative stress induction as well as in immune and inflammatory responses, suggesting a role for leptin/LEPR signal as a promising molecular basis underlying ATLI onset and progress. In this work, we focus on the *LEPR* gene which may represent a potentially gene implicated in ATLI. The LEPR protein, encoded by *leptin receptor* (*LEPR)* gene, is turned on by leptin binding, and is expressed in almost all tissues in human. This receptor belongs to type I cytokine receptor superfamily. So far, six alternatively spliced isoforms of LEPRa-f have been identified. Among them, only the long form, LEPRb, can transmit intracellular leptin signaling (Lago et al., 2008). The human *LEPR* gene contains a number of SNPs, and many of such polymorphisms in this gene have been shown to be significantly associated with obesity-related disorders (Yang and Niu, 2018; Nowzari et al., 2018) and various types of human malignant tumors (Abdu Allah et al., 2018; Méndez-Hernández et al., 2017). However, the potential associations between genetic variants of *LEPR* and susceptibility to ATLI have yet to be studied.

As far as we know, the present study is the first attempt to perform an association analysis between *LEPR* genetic variants and predisposition and clinical traits of ATLI in Western Chinese Han TB patients. We discovery that rs2025804 within *LEPR* gene was associated with susceptibility to liver injury caused by INH and RIF co-administration, and rs10889551, rs2025804 and rs2104564 were related to the peripheral levels of CRP of ATLI individuals among Western Chinese Han population. Furthermore, ATLI was identified in 118 out of 745 TB patients undergoing cotreatment with INH and RIF, with an occurrence rate of 15.84% in this study. This incidence rate is modest compared to incidence rates of previous studies (3% - 28%). The differences in ATLI incidence rates observed among studies might be due to variations in

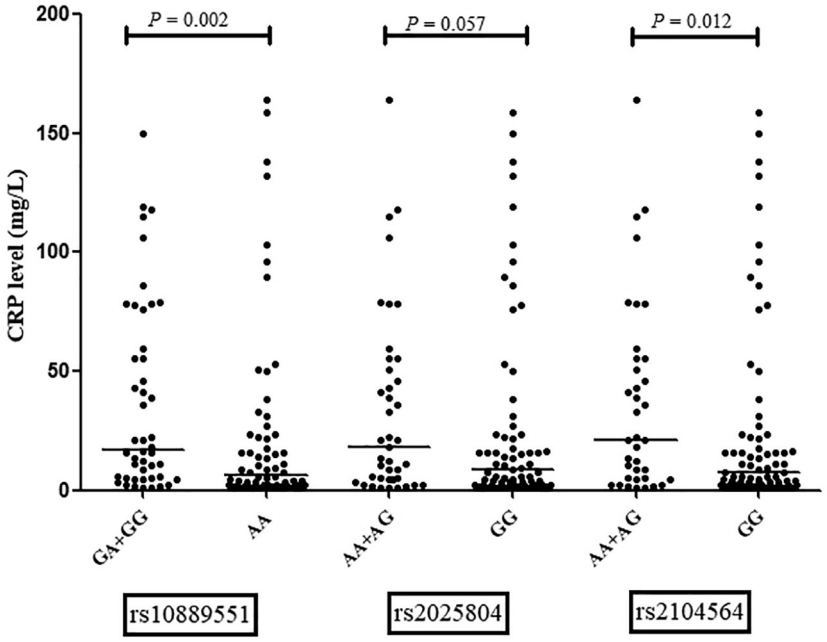

Fig. 3. The differences in CRP levels (mg/L) among ATLI individuals under the dominant pattern of rs10889551, rs2025804 and rs2104564. In ATLI patients, subjects carrying minor allele-containing genotypes in rs10889551, rs2025804 and rs2104564 loci had higher CRP levels in comparison with those patients harboring homozygous major alleles (rs10889551: 19.40 mg/L *vs.* 6.53 mg/L, $p = 0.002$; rs2025804: 20.90 mg/L *vs.* 8.41 mg/L, $p = 0.057$; rs2104564: 22.00 mg/L *vs.* 7.52 mg/L, $p = 0.012$).

study settings, definitions of hepatotoxicity utilized, ethnicity, genetic background, and sample size. Hence, a larger cohort study among patients taking anti-TB treatment is urgently needed to provide the more accurate and comprehensive information on the ATLI morbidity.

Previous studies investigating the associations between *LEPR* variation rs2025804 and breast cancer subtypes in North Carolina (Nyante et al., 2011) as well as diabetes mellitus type 2 in a Kazakh population (Sikhayeva et al., 2017) reported negative findings. Moreover, earlier studies have found that rs2025804 G allele is associated with enhanced obesity risk in Caucasian (Fox et al., 2007), Chinese (Chen et al., 2009) and Native Americans populations (Traurig et al., 2012). *Via* this pilot prospective investigation, we first show that *LEPR* polymorphism, rs2025804 A allele, significantly contributes to ATLI development in Western Chinese Han TB patients. SNP rs2025804 A allele was significantly enriched in patients with ATLI and exhibited a 1.73-fold and 1.64-time higher risk for ATLI under the dominant and additive genetic pattern. Through searching in the RegulomeDB (http://regulome.stanford.edu/) and HaploReg v4.1 (http://www.broadinstitute.org/mammals/haploreg) databases, we annotate that SNP rs2025804 is located at some motif regions where refer to some specific transcription factors (shown in Supplementary Table S2). In addition, the expression quantitative trait locus (eQTL) analysis based on the public GTEx database (http://www.gtexportal.org/) shows that SNP rs2025804 was significantly correlated with the expression of *LEPR* in adipose tissue ($p < 0.001$) (shown in Supplementary Fig. S2). These findings indicate the potential biological effect of *LEPR* SNP rs2025804 on ATLI onset, and this effect might be mediated by its effect on the gene expression of *LEPR*. Further researches are warranted in order to explore the detail mechanisms.

ATLI is a complicated disease involving multiple pathogenic genes, and thus the contribution of a single SNP to the occurrence of disease is limited. Therefore, in this investigation we assess the associations between haplotype and multi-loci interaction models with ATLI predisposition. The researches have previously revealed that haplotype analysis-based association study provides more convincing evidence than result from a single marker analysis (Morris and Kaplan, 2002). In this work, haplotype AA comprising of rs2025804 and rs2104564 showed a 1.58-fold increased risk for ATLI when compared with the GG haplotype, which finding is consistent with the individual rs2025804 association analyses. Recently, studies suggest that the disease onset is closely related to interactions among multiple genetic loci (Fan et al., 2016). We applied GMDR method to identify interaction models among *LEPR* polymorphisms that correlated with ATLI susceptibility. Nevertheless, no statistically significant interaction models among six SNPs were determined.

Studies have already found that the important contribution of the immune and inflammatory response to the development and progress of ATLI (Ramappa and Aithal, 2013; Chen et al., 2015; Holt and Ju, 2006). In this study, an increase in peripheral levels of CRP was observed in subjects with minor alleles of G and A of *LEPR* polymorphisms rs10889551 and rs2104564, among individuals experienced ATLI ($p = 0.002$ and $p = 0.012$, respectively). In the meantime, the ATLI relevant SNP rs2025804, its genotypes (AA + AG) tended to present relatively higher CRP level ($p = 0.057$). CRP plays a critical role in the innate immunity and is synthesized primarily in the liver in response to certain pro-inflammatory cytokines. CRP, as an inflammatory biosignature and acute phase protein, is considered to be a key circulating biomarker indicating acute inflammation response in humans (Felger et al., 2018). Multiple studies have recently elucidated that leptin signal plays a major role in immune reactions and inflammatory responses. In the autoimmune and non-autoimmune inflammation, the nature of the pro-inflammatory activity of leptin has been identified (La Cava, 2017). Furthermore, there are also wide-ranging reports with respect to leptin signal's role in the release of inflammatory mediators (Lafrance et al., 2010; Zarkesh-Esfahani et al., 2004; Wei et al., 2019; Fu et al., 2017). Such as, leptin could stimulate the production of pro-inflammatory cytokines of IL-6 and IL-8, and IL-18 in human dental pulp fibroblasts and in RAW 264.7 cells, respectively (Wei et al., 2019; Fu et al., 2017). Of note, Yoshino T et al. have described that the serum leptin levels were obviously elevated and were positively associated with CRP levels in patients with rheumatoid arthritis (Yoshino et al., 2011). In-silico analysis results suggest that, SNP rs10889551 lies in the motif regions where may bind with several certain transcription factors and evidently associates with the *LEPR* gene expression levels in adipose tissue ($p < 0.001$); rs2104564 locates at a locus with transcription factor binding potential as predicted by HaploReg v4.1 (primary information depicted in Supplementary Table S2 and Supplementary Fig. S2). The above literature reports and bioinformatics analysis indicate that functional candidates rs10889551, rs2025804 and rs2104564, may be greatly associated with inflammatory response in patients with ATLI through affecting the leptin/LEPR pathway expression and/or activity, however, the specific molecular mechanisms require further

investigation. On the other hand, given the cytokines released during the immune and inflammatory processes following ATLI onset could determine the outcome of this condition (Holt and Ju, 2006), *LEPR* polymorphisms and CRP possess a potential prognostic value for ATLI. This hypothesis requires the additional prospective study to confirm.

We also investigated associations between intron variants of *LEPR*, rs10889551 (A > G) and rs2104564 (G > A), and predisposition to ATLI, but these associations disappeared after multiple tests correction. Another intronic SNP, rs7413467 (G > A), and two exonic SNPs, rs1137101 (G > A, Gln223Arg) and rs1805134 (A > G, Ser343Ser), had no nominal association with ATLI predisposition in this study. These conclusions, however, are based on a small sample size and require careful interpretation. There are some limitations in our study which include limited SNP were analyzed in this study, thus the relationships between *LEPR* genetic polymorphisms and ATLI risk should be interpreted with caution. Further replication studies using larger population sample from other ethnic groups are needed to confirm the present findings.

In conclusion, we reveal that rs2025804 A allele may increase the risk of ATLI in Western Chinese Han population, suggesting that it might be used to identify patients with TB who are at risk of developing ATLI. In addition, we show that among ATLI patients, individuals carrying minor allele-containing genotypes in rs10889551, rs2025804 and rs2104564 loci have higher CRP levels and elevated inflammatory responses, suggesting that they may impact the host inflammatory responses against liver damage caused by anti-tuberculosis treatments. *In vitro* and *in vivo* experiments are required to explore the mechanisms of leptin/LEPR pathway in ATLI development and progression.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103970.

Declaration of Competing Interests

All authors declare that they have no competing interests.

Acknowledgements

This work was funded by the National Natural Science Foundation of China [Grant 81672095]; National Science and Technology Major Project of China [Grant 2018ZX10715003-001]; University - City Science and Technology Cooperation Project of Sichuan University & Panzhihua City [2018CDPZH-13].

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