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Clinical significance of lnc-AC145676.2.1-6 and lnc-TGS1-1 and their variants in western Chinese tuberculosis patients

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ABSTRACT

Objectives: Tuberculosis (TB) remains a global public health problem. Recent studies have implicated long non-coding RNAs and their variants as possibly playing important roles in TB. The aim of this study was to assess the clinical relevance of lnc-AC145676.2.1-6 and lnc-TGS1-1 and their variants in a western Chinese population.

Methods: This case–control study included 467 TB patients and 473 healthy controls from West China Hospital. The expression levels of lnc-AC145676.2.1-6 and lnc-TGS1-1 were analyzed by reverse transcriptase quantitative real-time PCR. Single-nucleotide polymorphism genotyping was performed using a custom-designed 2 × 48-Plex SNPscan kit.

Results: It was observed that lnc-AC145676.2.1-6 and lnc-TGS1-1 expression levels were both obviously down-regulated in TB patients. In addition, a lower expression level of lnc-TGS1-1 was associated with the presence of thrombocytopenia in TB patients during anti-TB treatment, and the homozygous CC genotype of rs4737420 correlated with a decreased risk of leukopenia, compared with individuals with the T allele (TT/CT genotype), in the dominant mode.

Conclusions: For the first time, potential TB-associated promoting effects were identified for the decreased expression levels of lnc-AC145676.2.1-6 and lnc-TGS-1, while lnc-TGS1-1 and its variant rs4737420 may be predictive indicators of anti-TB drug-induced adverse drug reactions. Larger validation studies on different populations are warranted to confirm these findings.

Introduction

Tuberculosis (TB) is one of the top 10 causes of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. The World Health Organization reported that there were an estimated 10.0 million incident cases of TB and approximately 1.6 million individuals who died of TB worldwide in 2017 (World Health Organization, 2018). Humans are threatened by the high prevalence of HIV (Pawlowski et al., 2012) and the emergence of extensively drug-resistant TB (Walker et al., 2015). Of note, approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB), while the clear majority of them remain in latent infection status without clinical signs and symptoms, which indicates that a genetic predisposition may play an important role in the occurrence of TB. Although considerable advances have been achieved in recent years (Dheda and Maartens, 2016), the genetic and molecular mechanisms underlying this disease remain largely unclear.

In recent years, large-scale studies have revealed that three-quarters of the human genome is capable of being transcribed into primary transcripts, producing a range of non-coding RNAs (Djebali et al., 2012). Non-coding RNAs longer than 200 nucleotides are classified as lncRNAs, which have defective open reading frames (Derrien et al., 2012). As evidence has accumulated, lncRNAs have been found to participate in extensive biological and cellular processes (Beermann et al., 2016; Chen et al., 2017; Atianand et al., 2017). The expression levels of many lncRNAs are considered to be strictly regulated in several human diseases, including cancers (Harries Lorna, 2012; Tano and Akimitsu, 2012) and infectious diseases (Wang et al., 2017a; Westermann et al., 2016). A recent study revealed that a lncRNA named NRON, which is highly expressed in primary CD4+ T lymphocytes, is of great

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importance in HIV-1 latency by specifically inducing Tat protein degradation (Li et al., 2016). Similarly, it is also thought that lncRNAs act as regulators of immunocytes and the host defense during MTB infection. Wang et al. (2015) revealed that lncRNA-CD244 is up-regulated in active TB patients, which mediates H3K27 trimethylation at *infg/tnfa* loci and suppresses interferon gamma (IFN-γ)/tumor necrosis factor alpha (TNF-α) expression in CD8+T cells. As reported, SOCS3, which is a critical negative regulator of cytokine, and its nearby lncRNA XLOC_012582, are highly expressed in B cells of active TB patients (Fu et al., 2017). Most of the reported studies indicate that the functions of lncRNAs in MTB infection are highly worthy of wider and deeper exploration.

Meanwhile, the number of association studies on single-nucleotide polymorphisms (SNPs) with regard to TB susceptibility and clinical phenotypes are on the rise. Thye et al. (2012) reported that rs2057178, a genetic locus on the Wilms' tumor 1 gene, conferred resistance to TB. Curtis et al. (2015) found that rs10956514 was associated with TB susceptibility by reducing the level of *ASAP1* expression, thus leading to impaired migration of MTB-infected dendritic cells. The latest research on European races found that rs557011, rs9271378, and rs9272785, located in the region of the HLA class II gene, contribute to the complex genetic risk of TB (Sveinbjornsson et al., 2016). Recently, the importance of SNPs located in lncRNAs as key regulators has been recognized (Zheng et al., 2016; Guo et al., 2016; Rautanen et al., 2016). Zheng et al. (2016) confirmed that a variation at rs11655237 in LINC00673 confers susceptibility to tumorigenesis via creating a target site for miR-1231 binding. In addition, as reported, a risk-associated variant of lncRNA is able to regulate the expression of itself in a cis-regulatory manner (Guo et al., 2016). Despite these advances, there is a lack of evidence describing the association between lncRNA polymorphisms and clinical traits and predisposition to TB.

China is under serious threat from TB, especially in western regions, and the incidence of adverse drug reactions (ADRs) to anti-TB drugs (ATD) is higher than 10% (Hu et al., 2018; Wu et al., 2016). In a previous study using microarray analysis performed by the present authors, it was found that the expression levels of lnc-AC145676.2.1-6 and lnc-TGS1-1 were significantly down-regulated in active TB patients. Therefore, it could be valuable to further investigate whether lnc-AC145676.2.1-6 and lnc-TGS1-1 and their genetic variants are involved in the development and progression of active TB. Thus, a case–control study was performed to assess the associations of the expression levels of lnc-AC145676.2.1-6 and its variant rs111352767 and the expression levels of lnc-TGS1-1 and its variant rs4737420 with the predisposition to TB diseases, clinical phenotypes, and ATD-induced adverse drug reactions (ATD-ADRs) in a western Chinese population.

Methods

Study subjects

This case–control study included 467 TB patients (TB group) and 473 healthy controls (HC group). All of them were consecutively recruited from West China Hospital of Sichuan University from September 2015 to June 2017. Eligible patients were newly diagnosed by two respiratory physicians, independently, on the basis of typical symptoms, bacteriological evidence (smear microscopy, culture, and TB-DNA), radiological findings of active TB, and appropriate responses to anti-TB chemotherapy. All patients were treated with a 6-month course of chemotherapy, including isoniazid, rifampicin, pyrazinamide, and ethambutol. The exclusion criteria for patients included the presence of an immune deficiency, other infectious diseases, other lung diseases, cancers, and pregnancy. In addition, those with clear indications of abnormal liver, renal, or hematological function before anti-TB treatment were also excluded from the study.

Healthy controls were enrolled from the Physical Examination Center of West China Hospital at the same time. They were asymptomatic with a normal erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, and chest X-ray. The healthy controls were matched with TB patients according to age and sex. The latent TB infection status of healthy controls was unknown. All participants were non-relatives.

Informed consent was obtained from each participant. The study was approved by the Clinical Trial and Biomedical Ethics Committee of West China Hospital.

Clinical phenotypes

Demographic and clinical data of the patients were obtained from the medical records system of West China Hospital. Patients were followed up for at least 6 months to assess ATD-ADRs during the course of chemotherapy. This study investigated hepatic, renal, and hematological toxicity caused by ATDs. Generally, patients with alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels ≥3 times the upper limit of normal (ULN) (120 IU/l) in the presence of hepatitis symptoms, including nausea, vomiting, abdominal pain, jaundice, and unexplained fatigue, or with ALT and AST levels ≥5 times the ULN (150 IU/l) in the absence of symptoms, were considered as presenting ATD-induced hepatotoxicity (ATDH) (Nahid et al., 2016; Saukkonen et al., 2006). An increase in total bilirubin ≥1.5 times the ULN (42 μmol/l) was regarded as hyperbilirubinemia (National Cancer Institute and National Institutes of Health, 2009). Acute kidney injury (AKI) was defined as a process that causes an abrupt reduction in kidney function, such as an absolute increase in serum creatinine (SCr) (≥26.4 μmol/l) from the reference SCr within 48 h, or a percentage increase in SCr of 1.5-fold above the reference within 7 days (Mehta et al., 2015). The definition of chronic kidney disease (CKD) was kidney damage (microalbuminuria, proteinuria, casts, and other manifestations) or the presence of a glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for ≥3 months (Mehta et al., 2015). Anemia (hemoglobin ≤80 g/l), leukopenia (leukocyte count $<2.0 \times 10^9$/l), and thrombocytopenia (platelet count $<75 \times 10^9$/l) were different forms of hematological toxicity (National Cancer Institute and National Institutes of Health, 2009).

Expression of lncRNAs

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood samples were collected, and total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA). RNA was then reverse-transcribed to cDNA using an Omniscript Reverse Transcription kit (Qiagen, CA, USA). Quantitative real-time PCR (qPCR) was then used to measure the relative expression levels of target lncRNAs. The qPCR consisted of 5 μl of SYBR Premix Ex Taq II (Takara, Dalian, China), 3.2 μl of water, 1 μl of cDNA template, and 0.4 μl of 10 μM forward and reverse primers. Performed on a LightCycler 480 Real-Time PCR System (Roche Diagnostics, NJ, USA), the qPCR was programmed as an initial denaturation at 95 °C for 30 s and 40 cycles of amplification by denaturing at 95 °C for 5 s, annealing at 63 °C for 30 s, and extending at 72 °C for 30 s. High-resolution melting curve analysis was used as the control for quantitative specificity. A cycle threshold (Ct) value was considered acceptable if it fell between 10 cycles and 33 cycles. The relative expression level of each lncRNA was normalized to the GAPDH gene and was calculated using the $2^{-\Delta\Delta}$Ct method.

Genotyping of SNPs

The gene lnc-AC145676.2.1-6 is located at position 20 834 on chromosome 7, with 3 exons, while lnc-TGS1-1 is located at position 56 806 154 on chromosome 8, with 2 exons. rs111352767 of lnc-AC145676.2.1-6 and rs4737420 of lnc-TGS1-1 were obtained from the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) and lncRNASNP-human databases (http://bioinfo.life.hust.edu.cn/lncRNASNP). Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The SNP genotyping work was performed using a custom-by-design 2×48-Plex SNPscan kit (Genesky Biotechnologies Inc., Shanghai, China), which was based on double ligation and multiplex fluorescence PCR, as described by Y in Yin et al. (2014). To guarantee the genotyping quality, ddH_2O was considered to be the negative control used in each reaction, and approximately 10% of the total samples were randomly selected to be genotyped in duplicate.

Statistical analysis

The independent sample *t*-test and the Mann–Whitney *U*-test were applied for continuous variable analyses according to the normality of the data. The Chi-square test was used to analyze categorical variables, and Fisher's exact test was employed when the expected count was less than 5. The statistical analyses mentioned above were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Associations between SNPs and the expression of lncRNAs, TB risk, phenotype traits, and drug adverse responses were determined based on the distributions of alleles and different genetic models using PLINK (Mishra et al., 2013). The strength of association was estimated with the odds ratio (OR) and 95% confidence interval (CI). Statistical significance was set at an alpha level of 0.05.

Results

General characteristics of the participants

The demographic and clinical characteristics of the study participants are described in Table 1. In general, no significant difference in age or sex was found between TB patients and healthy controls, indicating that both groups were frequency-matched for age and sex. As expected, the mean body mass index (BMI) value of the cases was less than that of the controls (19.54 kg/m^2 vs. 23.36 kg/m^2, $p < 0.001$), and the rate of smoking in the TB group was higher than that in the control group (41.33% vs. 34.25%, $p = 0.025$). On comparison of the baseline laboratory parameters between the two groups, the cases presented lower levels of hemoglobin and albumin, but increased absolute platelet and leukocyte counts (all $p < 0.001$). Notably, both the ESR and CRP levels were significantly higher in TB cases than in controls.

As shown in Table 1, this study ultimately enrolled 467 patients with TB, including 240 pulmonary TB patients (PTB, 51.39%), 64 extrapulmonary TB patients (EPTB, 13.71%), and 163 PTB combined with EPTB patients (34.90%). Among all cases, the positive rate was

Table 1
Demographic and clinical characteristics of the study participants.

Characteristics	TB ($n = 467$)	HC ($n = 473$)	*p*-Value
General information			
Age (years), mean ± SD	45.37 ± 17.00	46.03 ± 12.44	0.496
Sex, male/female	284/183 (60.81%)	269/204 (56.87%)	0.219
BMI (kg/m^2), mean ± SD	19.54 ± 3.12	23.36 ± 2.43	<0.001
Smoking, yes/no	193/274 (41.33%)	162/311 (34.25%)	0.025
Baseline laboratory parameters, mean ± SD, or median (interquartile range) values			
Erythrocytes ($\times 10^{12}$/l)	4.27 ± 0.82	4.80 ± 0.48	<0.001
Hemoglobin (g/l)	120.31 ± 24.57	145.60 ± 15.19	<0.001
Hematocrit (%)	0.37 ± 0.07	0.45 ± 0.04	<0.001
Platelets ($\times 10^9$/l)	258.77 ± 123.12	170.77 ± 46.92	<0.001
Leukocytes ($\times 10^9$/l)	9.64 ± 4.50	6.08 ± 1.26	<0.001
TBIL (μmol/l)	8.00 (5.95–12.40)	13.00 (10.70–16.95)	<0.001
ALT (IU/l)	11.00 (7.00–16.50)	22.00 (16.00–31.50)	<0.001
AST (IU/l)	18.00 (14.00–23.00)	23.00 (19.00–29.00)	<0.001
ALB (g/l)	35.41 ± 6.98	46.67 ± 2.64	<0.001
Urea (mmol/l)	4.05 (3.18–5.28)	5.14 (4.21–6.11)	<0.001
Creatinine (μmol/l)	60.05 (49.03–74.88)	75.00 (63.85–86.15)	<0.001
CRP (mg/l)	31.00 (7.67–81.60)	5.91 (3.13–8.79)	<0.001
ESR (mm/h)	55.00 (28.00–84.00)	19.19 (10.57–28.00)	<0.001
TB-DNA, positive/negative	247/199 (55.38%)		
Smear, positive/negative	132/303 (30.34%)		
Culture, positive/negative	16/116 (12.12%)		
IGRA, positive/negative	330/137 (70.66%)		
TB clinical subtypes, *n* (%)			
PTB	240 (51.39%)		
EPTB	64 (13.71%)		
PTB and EPTB	163 (34.90%)		
TB-related adverse drug reactions, *n* (%)			
ATDH	65 (13.92%)		
Hyperbilirubinemia	12 (2.57%)		
AKI	29 (6.21%)		
CKD	13 (2.78%)		
mAnemia	46 (9.85%)		
Leukopenia	12 (2.57%)		
Thrombocytopenia	20 (4.28%)		

TB, tuberculosis; HC, healthy controls; SD, standard deviation; BMI, body mass index; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IGRA, interferon-gamma release assay; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis; ATDH, anti-tuberculosis drug-induced hepatotoxicity; AKI, acute kidney injury; CKD, chronic kidney disease.

highest for the interferon-gamma release assay (IGRA, 70.66%), followed by the TB-DNA test (55.38%), smear microscopy (30.34%), and MTB culture (12.12%). The overall incidence rates of ADRs were also investigated. ADTH (13.92%) was found to be the most common ATD-ADR, while anemia (9.85%) and AKI (6.21%) were the major toxicities of the hematological and nephritic systems, respectively.

Expression levels of lncRNAs in relation to TB risk

The relative expression levels of lncRNAs are shown in Table 2 and Figure 1. lnc-AC145676.2.1-6 and lnc-TGS1-1 were both significantly down-regulated in TB patients compared with healthy controls (0.77 (0.31–1.27) vs. 1.39 (0.35–3.16), $p < 0.001$; 0.23 (0.08–0.58) vs. 1.17 (0.36–2.66), $p < 0.001$, respectively). This study of RT-qPCR in a large population showed accordance with the results of the microarray analysis, indicating that lnc-AC145676.2.1-6 and lnc-TGS1-1 may play important roles in the occurrence and development of TB.

lncRNA genetic polymorphisms in the TB and HC groups

The polymorphisms of rs111352767 and rs4737420 were successfully genotyped in 467 TB patients and 473 healthy controls. The genotype distributions of polymorphism rs111352767 and rs4737420 in the control group conformed to Hardy–Weinberg equilibrium, with minor allele frequencies (MAFs) of 0.240 and 0.308, respectively. The allele and genotype frequencies of SNPs in the TB and HC groups are shown in the **Supplementary Material** (Table S1 and Table S2). The minor allele (A allele) frequency of rs111352767 was 23.07% in the TB group and 24.84% in the HC group, and the frequency of allele T in rs4737420 was 29.44% in the TB group and 32.14% in the HC group, with statistical p-values of 0.368 and 0.206, respectively. Additionally, no significant differences in genotype frequencies were found between the TB and HC groups (all $p > 0.05$). Furthermore, genetic model analysis, including dominant and recessive models, demonstrated no significant differences between the TB and HC groups (all $p > 0.05$).

Association of the expression of lncRNAs and their variants

As mentioned above, the expression levels of the target lncRNAs were significantly down-regulated in TB patients, even though no statistical significance was found in the association between genetic polymorphisms and TB risk. Previous reports have indicated that lncRNA variants may in turn influence the expression of lncRNA (Guo et al., 2016). In this study, as shown in Table 3, both lnc-AC145676.2.1-6 and lnc-TGS1-1 were still down-regulated in TB cases grouped by the dominant model (all $p < 0.05$). In addition, among HC, individuals carrying allele A (genotype AA and AT) of rs111352767 expressed lower lnc-AC145676.2.1-6 transcript levels compared with homozygous allele T carriers (genotype TT) (1.02 (0.26–2.84) vs. 1.57 (0.44–3.36), $p = 0.021$). For all cases and controls, the expression levels of lnc-TGS1-1 in rs4737420 allele T carriers (genotypes TT and CT) were higher than levels in those without allele T (genotype CC) (0.66 (0.17–2.10) vs. 0.44 (0.13–1.52), $p = 0.042$).

Table 2
Relative expression levels of lncRNAs in TB patients and healthy controls.

lncRNAs	TB ($n = 467$)	HC ($n = 473$)	Fold-change	p-Value
lnc-AC145676.2.1-6	0.77 (0.31–1.27)	1.39 (0.35–3.16)	0.55	<0.001
lnc-TGS1-1	0.23 (0.08–0.58)	1.17 (0.36–2.66)	0.20	<0.001

TB, tuberculosis; HC, healthy controls.

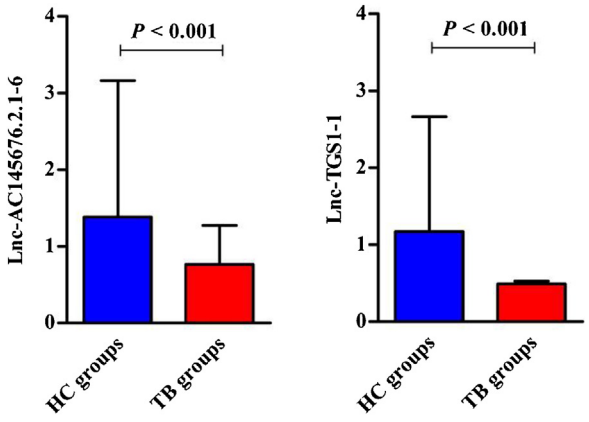

Figure 1. Relative expression levels of lncRNAs in tuberculosis patients and healthy controls.

Association between lncRNA expression and ATD-ADRs

Early reports showed that the occurrence of ATD-ADRs might be impacted by given genetic polymorphisms (Hu et al., 2018; Mishra et al., 2013). However, it appears that no research has reported whether the expression levels of lncRNAs are associated with ADT-ADRs. This study was novel in dividing all TB patients into two groups on the basis of the presence or absence of each adverse reaction (Table 4). Surprisingly, it was found that a lower expression level of lnc-TGS1-1 was associated with the presence of thrombocytopenia in TB patients during anti-TB treatment (presence vs. absence: 0.06 (0.04–0.32) vs. 0.25 (0.08–0.59), $p = 0.033$), but no associations were observed between relative lnc-AC145676.2.1-6 levels and the occurrence of adverse drug responses due to TB chemotherapy (all $p > 0.05$).

Association between lncRNA polymorphisms and ATD-ADRs

Therefore, an analysis was performed to determine whether the variants of lncRNAs influenced the occurrence of ADRs. Of note, the homozygous CC genotype of lnc-TGS1-1 rs4737420 was correlated with a decreased risk of leukopenia compared with those with the T allele (TT/CT genotype) in the dominant model (OR 0.20, 95% CI 0.04–0.93, $p = 0.023$) (Table 5). However, there was no association between lnc-AC145676.2.1-6 rs111352767 and ADT-ADRs (all $p > 0.05$), in accordance with the results of lnc-AC145676.2.1-6 relative expression levels.

Discussion

Thus far, the dominant point of view is that both environmental and genetic factors contribute to the occurrence and development of infectious diseases, while the importance of lncRNAs and their variants as regulators and biomarkers have not been recognized until recent years (Beermann et al., 2016; Wang et al., 2017a). The current study involved a preliminary discussion of two down-regulated lncRNAs in TB patients. The long intergenic non-coding RNA, lnc-AC145676.2.1-6, is located on chromosome 7, with a transcript size of 888 nucleotides. The non-coding RNA lnc-TGS1-1 is intronic on chromosome 8, with a transcript size of 651 nucleotides. Neither of these lncRNA has previously been reported to participate in the pathogenesis of any disease. Here, the differences in lnc-AC145676.2.1-6 and lnc-TGS1-1 expression

Table 3
Relative expression levels of lncRNAs in different variants.

lncRNAs	Genotypes	All	TB	HC	*p*-Value[a]
lnc-AC145676.2.1-6	AA + AT	0.83 (0.29–1.95)	0.77 (0.31–1.21)	1.02 (0.26–2.84)	0.026
	TT	1.01 (0.36–2.31)	0.75 (0.31–1.28)	1.57 (0.44–3.36)	<0.001
	p-Value[b]	0.050	0.866	0.021	
lnc-TGS1-1	TT + CT	0.66 (0.17–2.10)	0.22 (0.08–0.65)	1.40 (0.39–2.80)	<0.001
	CC	0.44 (0.13–1.52)	0.25 (0.07–0.51)	1.09 (0.30–2.48)	<0.001
	p-Value[b]	0.042	0.575	0.071	

TB, tuberculosis; HC, healthy controls.
[a] *p*-Value between cases and controls.
[b] *p*-Value of dominant model analysis.

Table 4
Relationships between the expression levels of lncRNAs and ATD-ADRs.

ATD-ADRs	lnc-AC145676.2.1-6	*p*-Value	lnc-TGS1-1	*p*-Value
ATDH				
Presence (*n* = 65)	0.72 (0.30–1.32)	0.987	0.24 (0.10–0.64)	0.530
Absence (*n* = 402)	0.77 (0.31–1.27)		0.25 (0.07–0.58)	
Hyperbilirubinemia				
Presence (*n* = 12)	0.72 (0.42–0.81)	0.885	0.21 (0.03–1.80)	0.984
Absence (*n* = 455)	0.77 (0.30–1.29)		0.24 (0.07–0.58)	
AKI				
Presence (*n* = 29)	0.66 (0.27–1.01)	0.417	0.11 (0.08–0.39)	0.194
Absence (*n* = 438)	0.77 (0.31–1.30)		0.25 (0.07–0.59)	
CKD				
Presence (*n* = 13)	0.73 (0.39–1.18)	0.956	0.17 (0.05–0.56)	0.555
Absence (*n* = 454)	0.77 (0.31–1.27)		0.24 (0.07–0.58)	
Anemia				
Presence (*n* = 46)	0.66 (0.30–1.24)	0.308	0.10 (0.04–0.38)	0.071
Absence (*n* = 421)	0.77 (0.31–1.27)		0.25 (0.08–0.59)	
Leukopenia				
Presence (*n* = 12)	0.45 (0.05–0.77)	0.177	0.20 (0.07–0.35)	0.431
Absence (*n* = 455)	0.77 (0.32–1.27)		0.24 (0.08–0.58)	
Thrombocytopenia				
Presence (*n* = 20)	0.44 (0.15–1.01)	0.114	0.06 (0.04–0.32)	0.033
Absence (*n* = 447)	0.77 (0.32–1.27)		0.25 (0.08–0.59)	

ATD-ADRs, anti-tuberculosis drug-induced adverse drug reactions; ATDH, anti-tuberculosis drug-induced hepatotoxicity; AKI, acute kidney injury; CKD, chronic kidney disease.

levels and SNPs between TB and HC groups were first explored and then the associations of those genetic characteristics with clinical phenotypes and ATD-ADRs in TB patients were evaluated.

In the current research, it was found that the expression levels of lnc-AC145676.2.1-6 and lnc-TGS1-1 were both obviously down-regulated in TB patients, implying that lnc-AC145676.2.1-6 and lnc-TGS1-1 may somehow reduce the risk of MTB infection, although no significant association was found between lnc-AC145676.2.1-6 rs111352767 (or lnc-TGS1-1 rs4737420) and the predisposition to TB diseases. Recently, lncRNAs functioning as competing endogenous RNAs (ceRNAs) involved in biological processes and disorders have been reported widely (Thomson and Dinger, 2016). Song et al. (2017) found that lncRNA-KRTAP5-AS1 and lncRNA-TUBB2A could serve as ceRNAs to regulate *CLDN4* expression in gastric cancer by competitively binding miR-596 and miR-3620-3p, respectively. From the Ensembl (Zerbino et al., 2018) and lncRNASNP2 (Miao et al., 2018) databases, it was found that lnc-TGS1-1 is able to bind with 25 microRNA (miRNAs) (**Supplementary Material**, Figure S1). Specifically, miR-143, a binding target of lnc-TGS1-1, is significantly increased under infection conditions, thus suppressing immune function via reducing downstream gene expression (Zhou et al., 2015; Zhao et al., 2014). In addition, SNP rs4737420 is located in the non-coding region of lnc-TGS1-1 and may impact the interactions between lnc-TGS1-1 and many miRNAs, which may cause lnc-TGS1-1 to lose the capacity to bind with miR-143. In the present study, the expression levels of lnc-TGS1-1 in allele T carriers (genotype TT and CT) were higher than those in subjects without allele T (genotype CC) in all subjects.

Meanwhile, lnc-AC145676.2.1-6 is able to interact with 45 miRNAs (**Supplementary Material**, Figure S2), several of which have been reported to correlate with mycobacterial infection, including miR-29a (Siddle et al., 2014; Fu et al., 2011; Sharbati et al., 2011), miR-150 (Ghorpade et al., 2012; Wang et al., 2017b), and miR-92a (Wang et al., 2018). Accumulated evidence implies that dysregulated miR-29a is critical to the pathogenesis of TB. A previous study (Siddle et al., 2014) reported that miR-29a is significantly up-regulated after infection with MTB and inhibits target gene expression mainly involved in cytokine–cytokine receptor interactions and toll-like receptor (TLR) signaling pathways. In particular, *CXCL10* has been demonstrated to be significantly down-regulated by miR-29 upon MTB infection. The experimental study results, coupled with bioinformatics predictions and the results of previous studies, suggest that lnc-TGS1-1 and lnc-AC145676.2.1-6 may be able to act as miRNA sponges to interact with their candidate miRNAs and to participate in the occurrence and development of TB.

Clinically, long-course anti-TB combination regimens could lead to various types of ADRs, which could subsequently lead to therapy modification or discontinuation, treatment failure, and even death (Munro et al., 2007; Sharma et al., 2010). This study

Table 5
Relationships between SNPs and ATD-ADRs in the dominant model.

ATD-ADRs	Rs111352767		*p*-Value[a]	OR (95% CI)	Rs4737420		*p*-Value	OR (95% CI)
	AA + AT (*n* = 188)	TT (*n* = 278)			TT + CT (*n* = 238)	CC (*n* = 229)		
Anemia, *n* (%)	15 (7.98)	31 (11.15)	0.260	1.45 (0.76–2.76)	20 (8.40)	26 (11.35)	0.285	1.40 (0.76–2.58)
Leukopenia, *n* (%)	6 (3.19)	6 (2.16)	0.695[a]	0.67 (0.21–2.11)	10 (4.20)	2 (0.87)	0.023	0.20 (0.04–0.93)
Thrombocytopenia, *n* (%)	7 (3.72)	13 (4.68)	0.619	1.27 (0.50–3.24)	11 (4.62)	9 (3.93)	0.712	0.84 (0.34–2.08)
Hyperbilirubinemia, *n* (%)	4 (2.13)	8 (2.88)	0.839[a]	1.36 (0.41–4.59)	3 (1.26)	9 (3.93)	0.068	3.21 (0.86–12.00)
ATDH, *n* (%)	29 (15.43)	36 (12.95)	0.449	0.82 (0.48–1.38)	37 (15.55)	28 (12.23)	0.300	0.76 (0.45–1.28)
AKI, *n* (%)	8 (4.26)	21 (7.55)	0.148	1.84 (0.80–4.24)	16 (6.72)	13 (5.68)	0.640	0.84 (0.39–1.78)
CKD, *n* (%)	5 (2.66)	8 (2.88)	0.888	1.08 (0.35–3.37)	6 (2.52)	7 (3.06)	0.725	1.22 (0.40–3.68)

ATD-ADRs, anti-tuberculosis drug-induced adverse drug reactions; OR, odds ratio; CI, confidence interval; ATDH, anti-tuberculosis drug-induced hepatotoxicity; AKI, acute kidney injury; CKD, chronic kidney disease.
[a] Indicates *p*-value adjusted by Fisher's exact test. Only the data of presenting ATD-ADRs was displayed and compared by Chi-square test.

found a 13.92% cumulative incidence of ATDH. As the leading ATD-ADR, the incidence rate of ATDH in this study was moderate compared with those found in other studies, which have varied from 2.55% to 36% due to different definitions, regimens, genetic heterogeneities, and sample sizes (Yamada et al., 2010; Shang et al., 2011; Kumar et al., 2010). Meanwhile, it is of note that the incidence of hematological toxicity has also posed non-negligible challenges to clinical medication safety (Nagayama et al., 2004). Here, three different forms of hematotoxicity were reported, covering anemia, leukopenia, and thrombocytopenia, with occurrence rates of 9.85%, 2.57%, and 4.28%, respectively. Although the decreases in leukocytes and thrombocytes were less frequent compared with anemia, they were more threatening and lethal.

Over the past decades, a number of studies have demonstrated the associations between genetic variants and susceptibility to ATD-ADRs (Chen et al., 2015), while no studies have reported whether lncRNA expression levels are associated with ADT-ADRs. The present study was novel in investigating the potential associations between lncRNA expression levels, their polymorphisms, and the incidence of ATD-ADRs. In TB patients, it was intriguingly discovered that a lower expression level of lnc-TGS1-1 was associated with the presence of thrombocytopenia, while the homozygous CC genotype of rs4737420 was correlated with a decreased risk of leukopenia compared with those with the T allele (TT/CT genotype) in the dominant model. Although the mechanism is still not clear, this finding may still provide a clue to screen out high-risk populations and to reduce adverse reactions. Studies with larger sample sizes and varied populations are needed to validate the results and to reveal more genetic risk factors associated with ATD-ADRs.

Although it provides a comprehensive illustration of the profiles of clinical phenotypes, ATD-ADRs, and genetic risk factors in a western Chinese population, this study has limitations. First, the significance of lnc-AC145676.2.1-6 and lnc-TGS1-1 expression in TB patients was determined and their potential functions predicted, but direct experiments to verify these results are lacking. Second, only one SNP was studied for each lncRNA and this failed to identify significant differences between cases and controls.

In conclusion, in this study, the potentially TB-associated promotive effect was first identified for the decreased expression levels of lnc-AC145676.2.1-6 and lnc-TGS1-1. Furthermore, it was observed that the expression levels of lnc-TGS1-1 and the variant rs4737420 may be predictive indicators of ATD-induced thrombocytopenia and leukopenia, respectively. Larger validation studies on different populations are warranted to confirm these findings.

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Ethical approval

Informed consent was obtained from each participant. This study was approved by the Clinical Trial and Biomedical Ethics Committee of West China Hospital.

Conflict of interest

None declared.

Author contributions

Hao Bai and Qian Wu wrote the main manuscript and fully participated in all experiments. Xuejiao Hu, Tao Wu, Jiajia Song, Tangyuheng Liu, Zirui Meng and Mengyuan Lv participated in the acquisition of data. Xiaojun Lu and Xuerong Chen participated in the analysis and interpretation of data. Yanhong Zhou and Binwu Ying designed the study. All authors made substantial contributions to writing and revising the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.04.018.

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