Original Article

Influence of *HNF4α* and *HNF4α-AS1* gene variants on the risk of anti-tuberculosis drugs-induced hepatotoxicity

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Background: The most serious and common complication of the medication recommended by World Health Organization (WHO) for tuberculosis (TB) is anti-tuberculosis drugs-induced hepatotoxicity (ATDH). Pregnane X receptor (PXR) is a key factor of ATDH, while Hepatocyte nuclear factor 4α (HNF4α) and hepatocyte nuclear factor 4 alpha-antisense-1 (HNF4α-AS1) may have co-regulating relationship with PXR. This study aimed to explore whether the genetic variants of *HNF4α* and *HNF4α-AS1* are associated with the predisposition of ATDH.

Methods: TB patients diagnosed in West China Hospital between December 2014 and April 2018 were enrolled. TagSNPs in *HNF4α* and *HNF4α-AS1* gene from the samples of the patients were genotyped with a custom-designed 2×48-plex SNP Scan™ Kit. The frequencies of the alleles, genotypes, genetic models and haplotype distribution of the variants were compared between the case and control groups. The association between SNP and ATDH risk was assessed by single factor logistic regression.

Results: Logistic regression analysis showed that none of the 15 genetic variants in *HNF4α* and *HNF4α-AS1* genes were significantly associated with susceptibility to ATDH in the Chinese Han population after Bonferroni correction.

Conclusions: A challenge has arisen to the promising application of SNPs in the *HNF4α* and *HNF4α-AS1* genes as genetic markers for ATDH, and further study is needed with a larger sample size.

Keywords: Hepatocyte nuclear factor 4α (HNF4α); hepatocyte nuclear factor 4 alpha-antisense-1 (HNF4α-AS1); anti-tuberculosis drugs-induced hepatotoxicity (ATDH); genetic polymorphism

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Introduction

Tuberculosis (TB) has remained a leading cause of morbidity and mortality worldwide, with a markedly pronounced severity in China, where the costs of TB have been reported as catastrophic to patients' families (1-3). Isoniazid, rifamycin, pyrazinamide, and ethambutol comprise the major regimen recommend for use by the World Health Organization (WHO) for patients with drug-susceptible TB (4). Although effective cure rate has reached about 95% under optimal conditions, the side effects of these regimens are not negligible, among which anti-tuberculosis drugs-induced hepatotoxicity (ATDH) is the most common and serious (5). The incidence of ATDH varies from 2% to 28% depending on different doses, schedules, and route of administration (5,6). The metabolites of isoniazid alone can induce hepatotoxicity; however, the incidence of hepatotoxicity increases significantly when isoniazid is co-administered with rifamycin (7). Although the mechanism of combination medication leading to enhanced hepatotoxicity has not been clearly addressed, a potential explanation is that rifamycin is a human-specific activator of pregnane X receptor (PXR)/nuclear receptor subfamily group I member 2 (NR1I2) (8,9). A nuclear receptor, *PXR* has been considered a master xenobiotic receptor coordinately regulating genes encoding drug-metabolizing enzymes (DMEs), such as cytochrome P450 enzymes (CYPs), conjugation enzymes, and transporters to essentially detoxify and eliminate xenobiotics (10). Due to its role in drug metabolism and transport, *PXR* was labeled a key factor in enhanced liver toxicity caused by rifamycin and isoniazid co-therapy (11). Unfortunately, Rifampicin and isoniazid co-therapy recommended by the WHO for TB patients was the main reason for ATDH, due to the effectiveness and economy of the co-therapy (4). With highly sensitive next-generation sequencing technology, transcriptional networks and upstream regulator analyses found that rifamycin is a stimulant of *PXR* and hepatocyte nuclear factor 4α (HNF4α). Hepatocyte nuclear factor 1 alpha-antisense-1 (HNF1α-AS1) and HNF4α-AS1 also regulate the expression and function of several drug-metabolizing cytochrome P450 (P450) enzymes, which also further impact P450-mediated drug metabolism and drug toxicity (12). Since both are involved in liver drug metabolism and drug toxicity by regulating cytochrome enzymes, these transcription factors (TFs) might be key regulators of a complex network of metabolism-associated pathways and result in unexpected drug-drug interactions (13).

The orphan nuclear receptor HNF4α is known as a master regulator of liver function (14,15). It is involved in many pathophysiological processes related to the development, proliferation, damage, and repair of hepatocytes, including hepatocellular carcinoma, non-alcoholic fatty liver disease, and drug-induced liver damage (16-18). Compared with the well identified functions of HNF4α, the underlying molecular mechanisms of HNF4α in drug-induced liver damage is still controversial. Overexpression of HNF4α sensitizes mice or primary hepatocytes to acetaminophen-induced liver injury (19). Meanwhile, degradation of HNF4α has been shown to aggravate steatosis and tumorigenesis in human livers induced by perfluorooctanoic acid and perfluorooctanesulfonic acid (20). Several TFs and co-regulators have been identified as potential specific partners for HNF4α (21). As a result, a possible mechanism of HNF4α in drug-induced hepatotoxicity is that it acts as an important regulator of coordinate *nuclear*-receptor-mediated response to xenobiotics (22,23). Transcriptional networks and upstream regulator analyses have shown HNF4α, PXR, and other TFs (NR1I3, RXRα, NF-κB) are hub regulators of the complex network of rifamycin relating drug-metabolizing enzymes (DMEs) and drug transporters (13,24). We speculate whether HNF4α participates in ATDH as a key gene in rifamycin-related drug metabolism pathways, similar to *PXR*.

Recent studies have suggested that long non-coding RNAs (lncRNAs) are also highly involved in physiological functions and diseases (25). It is worth mentioning that lncRNAs, especially neighborhood antisense lncRNAs, are involved in the expression or functions of TFs in gene regulation (26). HNF4α-AS1 is named based on its genomic location, which is the neighborhood region of HNF4α, and is transcribed in the opposite direction on the antisense strand. The *HNF4α* and *HNF4α-AS1* genes form a typical pair of coding and neighborhood antisense noncoding genes. Both *HNF1α-AS1* and *HNF4α* are highly expressed in liver. The similarity in tissue distribution might suggest functional connections between HNF4α and HNF4α-AS1 (27). A recent study found that *HNF4α-AS1* expression can be strongly activated by *HNF4α*, suggesting the expression regulatory net between the TF-lncRNA pair (28). According to increasing evidence, *HNF4α-AS1* has been found to regulate expression and function of several drug-metabolizing CYPs, which further impact CYP-mediated drug metabolism and drug toxicity (1,4). However, there is still no clear evidence showing the exact co-regulatory

mechanism of the TF-lncRNA pair in ATDH. So far, it can only be confirmed that both of them have a crossover with *PXR* in regulation of CYPs (13,24,28). Since *PXR* is a key factor of ATDH, the question arises of whether the TF-lncRNA pair participates in ATDH through interaction with *PXR*.

Due to the atypical symptoms and signs of ATDH, its early diagnosis and prevention is still a challenge (29). If ATDH occurs, it may be recommended to temporarily discontinue the drug for medical observation. If the patient can tolerate it, the combination drug will still be recommended, but rifampicin may be replaced with a combination of rifapentin and isoniazid, or a more expensive second-line drug combination (1,30). Factors contributing to ATDH include genetic, epigenetic, physiological, pathological, and environmental (24). Among them, genetic predisposition plays an important role in occurrence and progress of ATDH (5,31,32). Pharmacogenetics can guide drug treatment according to patient genetics. It has been extensively applied to various fields of medicine to prevent serious adverse drug reactions (33,34). Pharmacogenetics has provided 27 pairs of annotated variant-drug pairs for TB treatment, which are mainly associated with hepatotoxicity (35). The number of association studies between polymorphisms of key genes and susceptibility to ATDH is still on the rise (36-39). Meanwhile, pharmacogenetics researchers have explored the influence of *HNF4α* genetic variants on drug plasma concentration and susceptibility to adverse drug effects, including docetaxel-induced myelosuppression, imatinib-induced periorbital edema, and so on (40-44). Recently, a genome-wide association study (GWAS) identified that 4 single nucleotide polymorphisms (SNPs) of HNF4α were significantly associated with cytotoxicity in HepG2 cells after treatment with emodin (17). Accumulating evidence indicates that lncRNA polymorphisms may also be potential biomarkers used for early diagnosis, monitoring therapy response, and prognostic assessment including for TB (45,46). Since results of current pharmacogenomics studies on ATDH are still lacking consistency according to different races, drug dosages, and treatment protocol, the results may not be representative of a Chinese population infected with TB (31,32,47-49). Considering China's heavy burden of TB, further pharmacogenetic studies aiming to identify novel potential targets are needed in order to provide a better understanding of the potential mechanism of ATDH and optimize treatment outcomes.

To our knowledge, *HNF4α* and *HNF4α-AS1* are involved in liver functions. However, genetic polymorphisms of PXR have also been regarded to increase susceptibility to ATDH in different population, genetic polymorphisms of *HNF4α* and *HNF4α-AS1* have also been regarded to related to some metabolic diseases in different population (50,51), the correlation between genetic polymorphisms of *HNF4α* and *HNF4α-AS1* and predisposition of ATDH has not been elucidated yet (12,37-39,52). Pharmacogenetic study of *HNF4α* and *HNF4α-AS1* may be an acceptable tool for treatment optimization of anti-tubercular drugs. Therefore, the purpose of this study was to clarify whether *HNF4α-AS1* is involved in *PXR* regulation through bioinformatics, and to explore if the genetic variants contribute to the susceptibility of ATDH or clinical laboratory characteristics. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-2924).

Methods

Samples

The blood samples of this study were stored in the Bio-Bank of resources "Tuberculosis Researches" in the Department of Laboratory Medicine, West China Hospital, Sichuan University, China, as mentioned previously (37,53,54). According to the ATDH inclusion and exclusion criteria, there were a total of 118 samples in the ATDH group and 628 samples in the non-ATDH group (55,56). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval for this study was granted by the Institutional Review Board of the West China Hospital of Sichuan University (2014-198). The subjects of the study had already signed an informed consent form at the beginning of the whole study.

Candidate SNP selection and genotyping

TagSNPs located in *HNF4α* and *HNF4α-AS1* genomic regions were selected as mentioned previously with priority SNPs that may be related to the risk of ATDH or potential functional significance (37,57). The SNP genotyping work was conducted by the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) and custom-by-design 2×48-Plex SNP Scan™ Kit (Cat#: G0104, Gene Sky Biotechnologies Inc., Shanghai, China) as described previously (58). Thirty samples were selected for double-blind experiments randomly for repeatability and stability of genotyping.

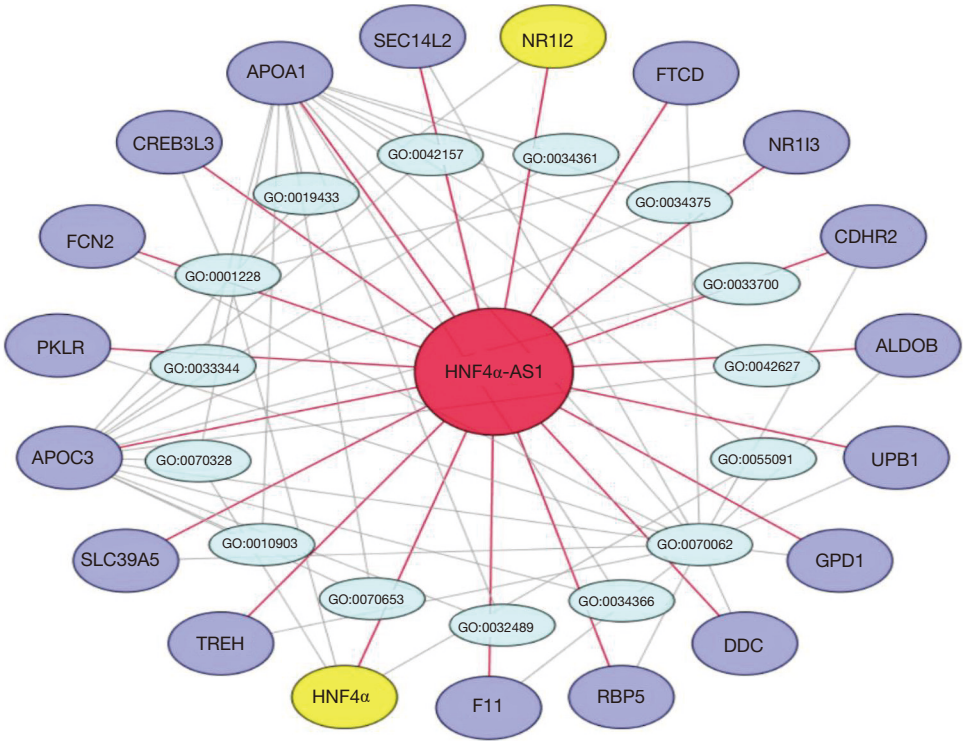

Figure 1 The gene annotation and function enrichment analysis of target genes of HNF4α-AS1. Gene annotation and function enrichment analysis further indicated HNF4α-AS1 may have transcriptional co-activator activity and/or RNA polymerase II transcriptional regulatory region sequence specific binding ability for PXR/NR1I2 (GO:0001228) and HNF4α (GO:0070653). HNF4α-AS1, hepatocyte nuclear factor 4 alpha-antisense-1; PXR, pregnane X receptor; NR1I2, nuclear receptor subfamily group I member 2; GO, Gene Ontology; HNF4α, hepatocyte nuclear factor 4α.

Statistical analysis

The data in the ATDH and non-ATDH groups were compared using independent *t*-test (continuous variables) or chi-square test (categorical variable) by SPSS version 17.0 (IBM Corp., Chicago, IL, USA). Associations between SNPs and the risk of ATDH were evaluated by Plink version 1.07. The LD and haplotype analysis were conducted by Haploview version 4.2. The SNP-SNP interactions were analyzed by Multifactor Dimensionality Reduction software (MDR; version 3.0.1). Schematic diagram was conducted by Cytoscape (version 3.7.1; https://cytoscape.org/). Two-sided values of $P<0.05$ were considered statistically significant (59).

Results

Preliminary bioinformatics analysis

We first used lncRNA and protein interaction gene co-expression matrix [multi experiment matrix (MEM)] to predict the target genes regulated by HNF4α-AS1, and found HNF4α-AS1 and *PXR* may have potential interactions (https://biit.cs.ut.ee/mem/index.cgi), as shown in Figure S1. Using the online gene annotation and function enrichment website DAVID software (https://david.ncifcrf.gov), we performed Gene Ontology (GO) function annotation and function enrichment analysis on potential target genes of HNF4α-AS1, and found that some TFs (*NR1I3*, *PXR*, and *HNF4A*, among others) had activity as transcriptional co-activator and/or specific binding ability with RNA polymerase II transcription regulatory region sequence (GO:0001228 and GO:0070653). A schematic diagram of *HNF4α-AS1* regulation of related TFs is depicted in *Figure 1*.

Participant demographic characteristics

In total, 746 TB patients were consecutively included, 118 in the ATDH group and 628 in the non-ATDH

Table 1 The distributions of allele and genotype frequencies of all the 15 SNPs

Gene	dbSNP	Allele (1/2)	Allele				Genotype		
			ATDH (1/2)	Non-ATDH (1/2)	OR (95% CI)	P value	ATDH (11/12/22)	Non-ATDH (11/12/22)	P value
HNF4α-AS1	rs6017335	C/A	102/134	544/712	0.996 (0.752–1.319)	0.979	19/64/35	128/288/212	0.234
	rs2425637	G/T	118/118	574/680	1.185 (0.896–1.565)	0.232	32/54/32	143/288/196	0.510
	rs2868094	C/A	62/174	409/845	0.736 (0.538–1.007)	0.054	10/42/66	61/287/279	0.070
HNF4α	rs2071197	G/A	101/135	557/693	0.930 (0.702–1.233)	0.616	20/61/37	126/305/194	0.707
	rs3212183	T/C	18/218	53/1,203	1.874 (1.077–3.261)	0.024*	2/14/102	3/60/565	0.063
	rs11574730	G/A	24/212	149/1,107	0.841 (0.533–1.326)	0.455	2/20/96	6/137/485	0.398
	rs6093978	C/T	78/158	399/855	1.058 (0.786–1.422)	0.709	9/60/49	66/267/294	0.223
	rs3212198	C/T	75/161	392/860	1.022 (0.757–1.378)	0.886	8/59/51	63/266/297	0.248
	rs3212200	T/C	55/181	261/989	1.151 (0.826–1.603)	0.403	3/49/66	27/207/391	0.172
	rs6103731	A/G	76/160	384/864	1.069 (0.793–1.440)	0.662	8/60/50	63/258/303	0.133
	rs2273618	T/C	96/140	445/807	1.244 (0.935–1.653)	0.132	17/62/39	85/275/266	0.147
	rs3212208	T/C	18/218	66/1,190	1.489 (0.866–2.557)	0.146	1/16/101	4/45/579	0.238
	rs3818247	T/G	97/139	458/796	1.213 (0.913–1.611)	0.182	18/61/39	88/282/257	0.265
	rs3746574	T/C	80/156	405/845	1.070 (0.797–1.436)	0.652	10/60/48	62/281/282	0.495
	rs6130615	T/C	85/151	545/705	0.728 (0.545–0.971)	0.030*	17/51/50	122/301/202	0.088

*, P<0.05. P value was calculated using logistic regression analysis. 1= the mutant allele; 2= the wild allele; 11= mutant homozygote; 12= heterozygote; 22= wild homozygote. SNP, single nucleotide polymorphism; dbSNP, SNP database; ATDH, anti-tuberculosis drug-induced hepatotoxicity; OR, odds ratio; CI, confidence interval; HNF4α-AS1, hepatocyte nuclear factor 4 alpha-antisense-1; HNF4α, hepatocyte nuclear factor 4α.

group. There was no difference in age (42.85±18.44 *vs.* 40.92±15.72; P=0.284) and gender (proportion of male 58.47% *vs.* 59.71%; P=0.801) between ATDH group and non-ATDH group.

SNP allele, genotype, genetic model, and haplotype analysis

Based on criteria mentioned above, 15 SNPs were selected and genotyped successfully for 99.9% of participants. All genotype frequencies of selected SNPs in the non-ATDH group followed the Hardy-Weinberg Equilibrium (HWE) law (P>0.05) (Table S1). The allele distributions and genotype frequencies of all 15 SNPs are presented in *Table 1*. For the rs3212183 locus, the proportion of T allele was 18/218 (7.62%) in the ATDH group and 53/1,203 (4.22%) in the non-ATDH group, compared with C allele (OR: 1.874; 95% CI: 1.077 to 3.261, P=0.024). For the rs6130615 locus, the proportion of T allele was 85/151 (36.01%) in the ATDH group and 545/705 (43.60%) in the non-ATDH group, compared with C allele (OR: 0.728; 95% CI: 0.545 to 0.971, P=0.030). However, occurrence of genotype of these two loci showed no significant difference (both P>0.05). For other SNPs, no difference of allele or genotype was found between the two groups (all P>0.05). We also conducted multiple testing by Bonferroni correction which requires a more stringent significance level. When Bonferroni correction was applied, none of the 15 SNPs were statistically significant.

We constructed three genetic models (dominant, recessive, and additive patterns) to compare the significance of each SNP. As shown in *Table 2*, rs3212183 in dominant model (OR: 1.989; 95% CI: 1.102 to 3.591; P=0.022) and additive model (OR: 1.765; 95% CI: 1.042 to 2.991; P=0.034) showed statistical significance between these two groups; rs6130615 in dominant model (OR: 0.649; 95% CI: 0.434 to 0.970; P=0.034) and additive model (OR: 0.734; 95% CI: 0.551 to 0.976; P=0.033) showed statistical significance between the two groups; rs2868094 in the

Table 2 Genetic models of related SNPs association with ATDH in TB patients

Gene	dbSNP	Dominant model		Recessive model		Additive model	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
HNF4α-AS1	rs6017335	1.209 (0.787–1.855)	0.386	0.749 (0.442–1.271)	0.284	0.996 (0.756–1.312)	0.979
	rs2425637	1.222 (0.787–1.897)	0.371	1.259 (0.805–1.969)	0.311	1.170 (0.894–1.531)	0.250
	rs2868094	0.631 (0.425–0.938)	0.023*	0.859 (0.426–1.729)	0.670	0.730 (0.532–1.004)	0.052
HNF4α	rs2071197	0.985 (0.644–1.506)	0.945	0.808 (0.480–1.358)	0.421	0.930 (0.703–1.233)	0.617
	rs3212183	1.989 (1.102–3.591)	0.022*	1.333 (0.147–12.04)	0.797	1.765 (1.042–2.991)	0.034*
	rs11574730	0.777 (0.471–1.281)	0.322	1.787 (0.356–8.964)	0.480	0.837 (0.527–1.328)	0.450
	rs6093978	1.243 (0.834–1.852)	0.284	0.701 (0.339–1.451)	0.339	1.058 (0.786–1.425)	0.708
	rs3212198	1.186 (0.797–1.763)	0.399	0.649 (0.302–1.395)	0.268	1.022 (0.756–1.381)	0.885
	rs3212200	1.316 (0.884–1.960)	0.175	0.577 (0.172–1.936)	0.374	1.157 (0.826–1.619)	0.397
	rs6103731	1.284 (0.862–1.910)	0.218	0.647 (0.301–1.39)	0.264	1.069 (0.792–1.441)	0.662
	rs2273618	1.497 (0.988–2.267)	0.056	1.071 (0.610–1.88)	0.810	1.238 (0.934–1.641)	0.137
	rs3212208	1.407 (0.781–2.532)	0.255	3.592 (0.593–21.73)	0.163	1.450 (0.860–2.444)	0.163
	rs3818247	1.407 (0.928–2.131)	0.107	1.103 (0.636–1.911)	0.728	1.210 (0.912–1.604)	0.186
	rs3746574	1.199 (0.804–1.788)	0.373	0.840 (0.418–1.691)	0.626	1.073 (0.794–1.451)	0.645
	rs6130615	0.649 (0.434–0.970)	0.035*	0.694 (0.400–1.203)	0.193	0.734 (0.551–0.976)	0.033*

*, P<0.05. P value was calculated using logistic regression analysis. SNP, single nucleotide polymorphism; dbSNP, SNP database; ATDH, anti-tuberculosis drug-induced hepatotoxicity; TB, tuberculosis; OR, odds ratio; CI, confidence interval; HNF4α-AS1, hepatocyte nuclear factor 4 alpha-antisense-1; HNF4α, hepatocyte nuclear factor 4α.

dominant model (OR: 0.631; 95% CI: 0.425 to 0.938; P=0.023) showed statistical significance between the two groups, but there was no statistical significance in the additive model. No genetic model was associated with susceptibility of ATDH in the SNPs after Bonferroni correction.

Haplotype was constructed to analyze additive association among selected SNPs with a frequency >0.05 and in strong LD state with one another by calculating the pairwise r^2 coefficient (r^2>0.80). As shown in Figure S2 and Table S2, no haplotype was associated with susceptibility of ATDH (all P>0.05).

SNP-SNP interactions associated with susceptibility to ATDH

A total of six models were established between all loci as shown in *Table 3*. A 3-point model composed of rs3212200, rs3212208, and rs3818247 was statistically different (P=0.010). The cross-validation consistency of this model was 9/10. The balance accuracy was 0.5876 and 0.5600 in training set and validation set, respectively. This result indicated that the joint factor obtained by this model was calculated through 9 crossover calculations, and the correct rate of distinguishing target population was about 57%. A 4-point model composed of rs3212200, rs6103731, rs3212208, and rs3818247 was also statistically different (P=0.010). In summary, there was a combined effect of these loci.

Relationship between genetic polymorphism and laboratory test indicators

Genetic polymorphism not only affects disease susceptibility, but also has a certain correlation with the clinical features of disease, which may affect the different clinical characteristics of individuals. In this study, the correlation between quantitative laboratory results of participants at baseline before treatment and genotype of the rs3212183 locus was analyzed. Patients with TT genotype had the highest erythrocyte sedimentation rate (ESR) 98.00 (50.00–120.00), and the ESR of patients with CC and CT genotype were 35.00 (21.00–58.75) and 46.00 (15.00–81.00),

Table 3 Interaction analysis on all 15 SNPs loci using MDR software

Model	Balance accuracy		CV consistency	P value
	Training group	Testing group		
rs3212200, rs3212208	0.5643	0.5249	8/10	0.179
rs3212200, rs3212208, rs3818247	0.5876	0.5600	9/10	0.010
rs3212200, rs6103731, rs3212208, rs3818247	0.5982	0.5619	7/10	0.010
rs2425637, rs1800963, rs3212200, rs2271618, rs6130615	0.6096	0.4990	3/10	0.623
rs6017335, rs2425637, rs1800963, rs3212200, rs2271618, rs6130615	0.6269	0.4910	3/10	0.828
rs6017335, rs2425637, rs1800963, rs6093978, rs3212200, rs2271618, rs6130615	0.6485	0.5059	4/10	0.377

SNP, single nucleotide polymorphism; MDR, Multifactor Dimensionality Reduction; CV, coefficient of variation.

respectively (Table S3). No correlation between laboratory indicators and genotype of the rs6130615 locus was found (Table S4).

Discussion

This study was undertaken to investigate the genetic influence of regulatory nuclear receptor (*HNF4α*) and its chaperone lncRNA (*HNF4α-AS1*) on susceptibility and clinical profiles of ATDH in a cohort of 746 Western Chinese patients with TB. Firstly, we explored whether *HNF4α-AS1* was involved in the transcriptional regulation network of *PXR*, which is a key factor in the mechanism of ATDH (11). Bioinformatics analysis suggested that *PXR* was a potential target gene of *HNF4α-AS1*. Gene annotation and function enrichment analysis further suggested *HNF4α-AS1* may modulate *PXR* and *HNF4a* expression through-RNA polymerase II (GO:0001228) and HNF4α (GO:0070653). Subsequently, we focused on analyzing the association between genetic polymorphism of both *HNF4α-AS1* and *HNF4α* with susceptibility to ATDH. As far as we know, this study was the first attempt to determine the association between *HNF4α* and *HNF4α-AS1* genetic variants with predisposition of ATDH.

In the study, all selected 15 SNPs were in HWE in the non-ATDH group. There were two SNPs nominally associated with ATDH, namely rs3212183 (P=0.024) and rs6130615 (P=0.030, adjusted for age and gender) (*Table 1*). As we tested the association for 15 SNPs, false positive was likely to occur merely by chance if we still adopted the nominal significance level of 0.05. Therefore, a more stringent significance level was required, we thus adopted Bonferroni correction. In the setting of this study, the significance level would be 0.05/15=0.0033. When Bonferroni correction was applied, none of the 15 SNPs were statistically significant, no matter in allele, genetic model, nor SNP-SNP interactions.

A member of steroid hormone receptor superfamily, *HNF4α* is a TF coded by the *HNF4α* gene. It is expressed in many tissues and is especially rich in liver. Consistent with the cell location, HNF4α has been suggested to have a role in several pathophysiological processes of hepatocytes, as well as some metabolic pathways such as glucose metabolism (16,17). Due to the physiological role of hepatocytes in xenobiotic detoxification and glycogen metabolism, mutations in coding and regulatory regions of *HNF4α* have been associated with drug side effect (periorbital edema caused by imatinib) and some metabolism-related diseases (type 2 diabetes) (60,61).

Previous studies have indicated that *HNF4α* is associated with type 2 diabetes, but the role of the variants of rs3212183, located in intron 3, in susceptibility was heterogeneous among different races (60,62). We investigated whether rs3212183 was associated with laboratory indicators related to liver function or metabolism of glucose. There was no association detected between genotypes and allele level of serum glucose in our study. It may be due to racial genetic differences between Finnish, Ashkenazi Jews, Pima Indians, and Chinese. As a result, its relative contribution to type 2 diabetes may differ between populations (63). It is also possible that we lacked the power to detect a subtle association due to the relatively small sample number (n=746). Although the sample size of the present study was fairly large in comparison with many other studies, it may nonetheless have low statistical power to detect variants with modest effects, especially allele (T)

for rs3212183 had a frequency of 0.762 in ATDH group and 0.422 in non-ATDH group. Thus, relationships between *HNF4α* genetic polymorphisms and ATDH risk should be interpreted with caution. Polymorphisms in the intron may affect messenger RNA (mRNA) stability and degradation, gene expression, and alternative splicing resulting in different protein isoforms (64,65). To some extent, the biological significance of SNPs located in intron is relatively difficult to verify. The genome-wide expression quantitative trait loci (eQTL) data from multiple tissues of major Genotype-Tissue Expression (GTEx) project databases is widely used in the biological function annotation function of SNPs (66). As a remedy, we annotated that SNP rs3212183 was significantly correlated with the expression of C20orf111 in whole blood (P=0.0005) through database HaploReg version 4.1 (https://www.broadinstitute.org/mammals/haploreg) (67).

The SNP rs6130615 is located in 3' untranslated region (3'-UTR) of *HNF4A* gene. Previous studies showed that patients with mechanical heart valves with CC genotype of rs6130615 had an 8.4-fold increased risk of bleeding during warfarin treatment (68). A reasonable explanation was that *HNF4α* mutation may result in vascular endothelial growth factor (VEGF) dysfunction. As VEGF is a well-known protein involved in vascular formation, VEGF dysfunction could lead to bleeding complications (69,70). Polymorphisms in the rs6130615 locus have also been reported in association with increased severity of anemia when treated with docetaxel. The biological effects of these variants may play a role in the interpatient variability in docetaxel pharmacokinetics (71). As stability and transport of mRNA transcripts are dependent on a properly configured 3'-UTR, we speculated that mutation at this locus would cause dysfunction of the protein by affecting gene expression and/or secondary structure of mRNA (64,65). We searched online StarBase database (https://starbase.sysu.edu.cn) to predict through bioinformatics and found miR-122 is a candidate miRNA for the 3'-UTR region mRNA of *HNF4α*. In multiple studies, it has been considered that miR-122 is related to drug-induced hepatotoxicity caused by isoniazid (72,73). Whether the mutation of *HNF4α* in the 3'-UTR region participates in ATDH by changing the interaction with miRNA is worth exploring. We also retrieved the genomic eQTL database and found rs6130615 was an eQTL for *SERINC3* gene expression in whole blood (P=0.00007) (67). Unfortunately, our study did not find any correlation between the genotype of this locus and blood cell count.

A neighbor antisense lncRNA gene of the human *HNF4α* gene, HNF4α-AS1 is located at human chromosome level with a length of 17.96 kb, containing 4 exons and 3 introns (12). Clues have indicated that expression regulatory net between the TF-lncRNA pairs is elevating (27,28,71). A haplotype constructed by rs6130608-rs2425637 has been reported to be correlated with the risk of metabolic syndrome in French-Canadian youth (74). Another study reported that polymorphisms of rs2425637 were significantly associated with type 2 diabetes at either allele or genotype level in Chinese people (75). Thus, as well as rs3212183 in *HNF4α*, the risk of rs2425637 polymorphisms contributed to type 2 diabetes might also be population specific. From the perspective of HNF4α-AS1 and HNF4α synergistic regulation of ATDH, no pharmacogenetic effects has ever been reported. We retrieved online website (https://bioinfo.bjmu.edu.cn/mirsnp/search/) to search potential functional SNPs with strong LD with rs2425637, and found no valuable clue for the biological significance of the variant (data not shown).

There were several strengths to our study: (I) participants were recruited from the West China Hospital, which the highest quality medical center in western China, to ensure the surveillance of ATDH with strict criteria to avoid misclassification. (II) The laboratory for testing is certified by the American Association of Pathologists to ensure all laboratory data had good quality and reliability. Our research also had some limitations. In future, to achieve significance, a larger sample size would be required.

We should also identify the association between the ATDH and performed functional verification tests *in vitro* and *vivo*.

We concluded that genetic polymorphisms of the *HNF4α* and *HNF4α-AS1* genes showed no significant associations with susceptibility to ATDH in the present Chinese Han population. Therefore, they did not appear to be major determinants for ATDH.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval for this study was granted by the Institutional Review Board of the West China Hospital of Sichuan University (2014-198). The subjects of the study had already signed an informed consent form at the beginning of the whole study.

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Figure S1 Interaction of HNF4α-AS1 with PXR (NR1I2). HNF4α-AS1, hepatocyte nuclear factor 4 alpha-antisense-1; PXR, pregnane X receptor; NR1I2, nuclear receptor subfamily group I member 2.

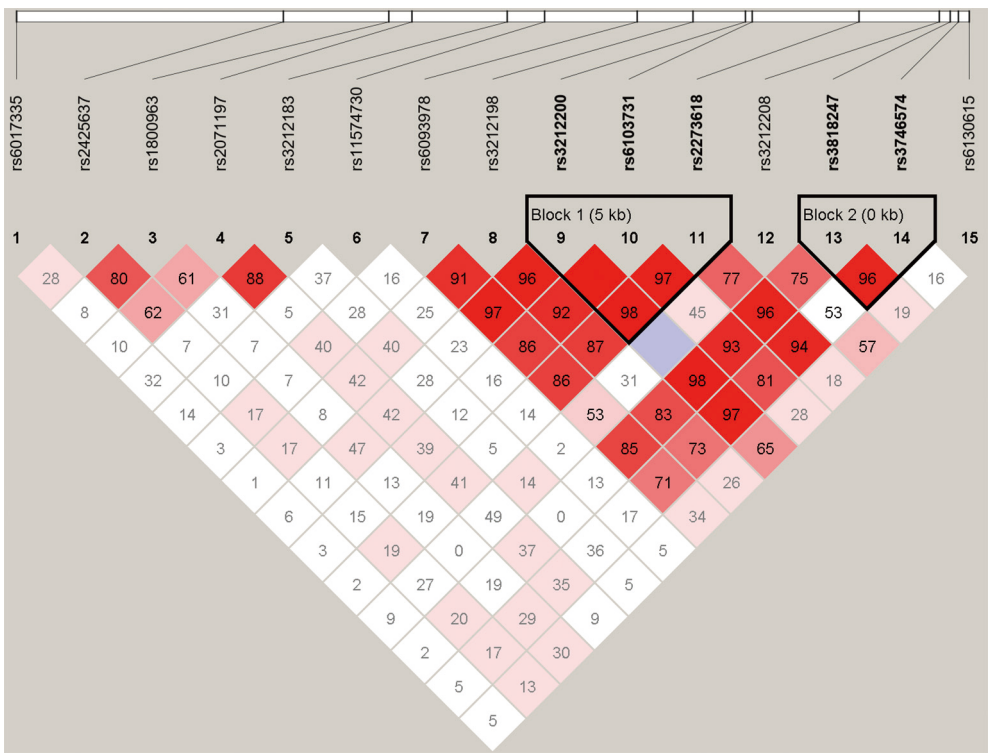

Figure S2 The loci of SNPs in the LD plots. SNP, single nucleotide polymorphism; LD, linkage disequilibrium.

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Table S1 Candidate single nucleotide polymorphism of HNF4α-AS1 and HNF4α

Gene	dbSNP	Allele	Position (GRCh38.p7)	MAF	MAF*	P^{HWE}
HNF4α-AS1	rs6017335	C>A	chr20:44382185	0.47	0.47	0.347
	rs2425637	G>T	chr20:44395409	0.49	0.45	0.360
	rs1800963	C>A	chr20:44400645	0.30	0.44	0.351
HNF4α	rs2071197	G>A	chr20:44401795	0.49	0.49	0.706
	rs3212183	T>C	chr20:44406498	0.04	0.09	0.128
	rs11574730	G>A	chr20:44408373	0.10	0.21	0.332
	rs6093978	C>T	chr20:44412951	0.33	0.43	0.145
	rs3212198	C>T	chr20:44415722	0.42	0.43	0.136
	rs3212200	T>C	chr20:44418289	0.24	0.34	0.120
	rs6103731	A>G	chr20:44418653	0.32	0.42	0.093
	rs2273618	T>C	chr20:44423930	0.50	0.45	0.445
	rs3212208	T>C	chr20:44427922	0.06	0.08	0.503
	rs3818247	T>G	chr20:44428840	0.35	0.46	0.568
	rs3746574	T>C	chr20:44429378	0.40	0.45	0.216
	rs6130615	T>C	chr20:44430797	0.39	0.47	0.549

*, MAF calculated by Haploview software in our study. MAF: MAF in 1000 Genomes (East Asia) https://www.ncbi.nlm.nih.gov/snp; P^{HWE}: P value of HWE. HWE was assessed by the χ^2 goodness-of-fit test based on the genotype distributions in this study. HNF4α-AS1, hepatocyte nuclear factor 4 alpha-antisense-1; HNF4α, hepatocyte nuclear factor 4α; dbSNP, single nucleotide polymorphism database; MAF, minor allele frequency; HWE, Hardy-Weinberg Equilibrium.

Table S2 Analysis of haplotypes with the risk of ATDH

Gene	SNP	Haplotype*	Frequency	P value
HNF4α	rs3212200:rs6103731:rs2273618	TAT	0.632	0.173
		CGC	0.211	0.373
		TGC	0.094	0.765
		CGT	0.058	0.056
	rs3818247:rs3746574	TT	0.620	0.222
		GC	0.318	0.550
		GT	0.055	0.106

*, ratio is shown by CC frequencies. ATDH, anti-tuberculosis drug-induced hepatotoxicity; SNP, single nucleotide polymorphism; HNF4α, hepatocyte nuclear factor 4α.

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Table S3 Analysis on the correlation between laboratory test indexes and genotype of rs3212183 locus

Laboratory indicators	rs3212183			P value
	CC	CT	TT	
RBC (10^{12}/L)	4.30±0.70	4.13±0.64	4.29±0.39	0.126
Hb (g/L)	122.86±20.98	125.82±18.65	126.00±12.22	0.446
Hct (%)	0.36±0.07	0.37±0.06	0.36±0.03	0.461
PLT (10^9/L)	240.91±20.98	254.00 (178.00–342.00)	145.55 (129.00–162.00)	0.070
WBC (10^9/L)	6.56 (4.77–7.78)	6.97 (5.25–8.83)	6.80 (5.46–8.14)	0.730
Neutrophil (%)	70.03±11.54	72.02±11.13	66.96±13.36	0.300
Lymphocyte (%)	18.20 (12.37–26.47)	16.30 (13.80–23.20)	25.00 (15.00–35.00)	0.120
Monocyte (%)	7.34±2.42	7.52±2.39	7.17±1.64	0.819
Neutrophils (10^9/L)	4.37 (3.27–6.11)	4.83 (3.30–6.54)	7.14 (5.89–8.39)	0.421
Lymphocyte (10^9/L)	1.20±0.57	1.34±0.77	3.73±3.69	0.261
Monocyte (10^9/L)	0.51±0.24	0.59±0.25	1.25±1.23	0.406
CRP (mmg/L)	11.00 (2.33–45.70)	11.20 (3.70–32.60)	64.05 (10.10–118.00)	0.157
ESR (mm/h)	35.00 (21.00–58.75)	46.00 (15.00–81.00)	98.00 (50.00–120.00)	0.037*
TBIL (μmol/L)	9.75 (7.50–13.85)	11.10 (7.40–15)	11.50 (9.80–20.70)	0.051
DBIL (μmol/L)	3.80 (2.52–6.95)	3.60 (2.30–5.60)	6.50 (3.30–7.70)	0.665
IBIL (μmol/L)	9.75 (7.50–13.85)	11.10 (7.40–15.00)	11.50 (9.80–20.70)	0.078
ALT (IU/L)	26.00 (15.25–38.00)	27.00 (12.00–38.00)	45.00 (27.00–47.00)	0.500
AST (IU/L)	26.40±8.99	27.00±8.34	28.00±7.81	0.483
TP (g/L)	69.02±8.99	67.94±9.15	66.17±9.59	0.494
ALB (g/L)	37.82±6.86	37.40±7.63	41.50±5.27	0.435
GLB (g/L)	31.18±7.00	30.52±6.37	24.66±3.62	0.086
Glucose (mmol/L)	5.71±2.16	5.78±2.01	5.17±0.50	0.823
Urea (mmol/L)	4.03 (3.09–5.20)	3.38 (2.70–4.90)	6.05 (5.31–11.05)	0.260
Crea (μmol/L)	57.40 (47.00–69.00)	62.00 (53.00–67.00)	80.10 (79.00–255)	0.620
Cystatin C (mg/L)	1.05±0.72	1.03±0.44	0.83±0.23	0.787
Uric acid (μmol/L)	327.00±157.08	324.85±147.58	314.70±117.56	0.979
TG (mmol/L)	1.01 (0.82–1.38)	0.95 (0.78–1.12)	1.57 (1.17–1.61)	0.626
CHOL (mmol/L)	3.96±1.05	4.10±1.40	3.84±1.02	0.647
HDL (mmol/L)	1.14±0.46	1.23±0.51	1.17±0.50	0.355
LDL (mmol/L)	2.28±0.79	2.36±1.02	2.32±0.62	0.788
ALP (IU/L)	100.00±79.30	91.90±26.20	96.60±14.60	0.458
GGT (IU/L)	46.00 (26.25–78.75)	36.00 (26.00–72.00)	68.00 (26.00–149.00)	0.572

*, P<0.05. RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; PLT, platelet; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; GLB, globulin; crea, creatinine; TG, triglycerides; CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALP, alkaline phosphatase; GGT, gamma glutamyl-transferase.

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Table S4 Analysis on the correlation between laboratory test indexes and genotype of rs6130615 locus

Laboratory indicators	rs6130615			P value
	CC	CT	TT	
RBC (10^{12}/L)	4.30±0.65	4.32±0.71	4.18±0.68	0.129
Hb (g/L)	122.10±20.07	123.76±20.19	119.16±22.73	0.083
Hct (%)	0.36±0.06	0.36±0.07	0.37±0.06	0.082
PLT (10^9/L)	236.00 (168.00–302.00)	232.00 (175.75–299.00)	235.00 (186.00–298.00)	0.625
WBC (10^9/L)	6.56 (4.77–7.78)	6.97 (5.25–8.83)	6.80 (5.46–8.14)	0.878
Neutrophil (%)	73.00 (66.00–79.00)	68.00 (59.20–77.70)	69.00 (63.05–69.70)	0.075
Lymphocyte (%)	18.20 (12.37–26.47)	16.30 (13.80–23.20)	25.00 (15.00–35.00)	0.157
Monocyte (%)	7.55±2.51	7.97±2.77	8.64±2.90	0.569
Neutrophils (10^9/L)	4.83 (3.31–6.54)	4.43 (3.27–6.31)	3.07 (2.48–3.67)	0.056
Lymphocyte (10^9/L)	1.20±0.58	1.42±1.03	1.11±0.37	0.301
Monocyte (10^9/L)	0.53±0.27	0.58±0.32	0.41±0.17	0.448
CRP (mmg/L)	11.80 (4.32–36.45)	13.40 (2.02–43.20)	10.79 (1.27–10.10)	0.188
ESR (mm/h)	45.50 (19.50–79.00)	35.00 (18.00–61.00)	54.00 (15.00–84.00)	0.496
TBIL (μmol/L)	10.50 (7.05–13.90)	10.30 (7.52–15.20)	11.95 (5.00–20.4)	0.821
DBIL (μmol/L)	3.60 (2.30–6.80)	3.70 (2.80–7.90)	2.20 (2.10–5.60)	0.195
IBIL (μmol/L)	6.20 (4.40–8.20)	4.65 (3.30–6.85)	8.40 (2.90–14.80)	0.301
ALT (IU/L)	26.00 (15.25–38.00)	27.00 (12.00–38.00)	45.00 (27.00–47.00)	0.115
AST (IU/L)	27.00 (17.00–31.00)	23.00 (17.00–29.00)	21.00 (15.00–27.00)	0.125
TP (g/L)	68.11±9.51	69.00±8.05	70.13±10.16	0.102
ALB (g/L)	37.49±7.25	37.95±7.80	38.80±6.69	0.678
GLB (g/L)	30.60±6.75	31.03±6.59	31.09±6.95	0.120
Glucose (mmol/L)	5.66±1.99	5.76±2.12	5.94±2.45	0.422
Urea (mmol/L)	4.03 (3.09–5.20)	3.38 (2.70–4.90)	6.05 (5.31–11.05)	0.605
Crea (μmol/L)	57.40 (47.00–69.00)	62.00 (53.00–67.00)	80.10 (79.00–255)	0.264
Cystatin C (mg/L)	1.05±0.72	1.03±0.44	0.83±0.23	0.213
Uric acid (μmol/L)	292.00±139.00	286.00±108.00	346.00±77.70	0.664
TG (mmol/L)	0.93 (0.76–1.30)	1.09 (0.85–1.45)	1.05 (0.82–1.52)	0.052
CHOL (mmol/L)	3.95±1.10	4.01±1.09	3.95±1.06	0.736
HDL (mmol/L)	1.15±0.47	1.15±0.46	1.12±0.48	0.840
LDL (mmol/L)	2.28±0.83	2.31±0.80	2.28±0.80	0.846
ALP (IU/L)	82.00 (62.00–102.75)	80.00 (67.00–99.00)	84.00 (64.75–102.25)	0.496
GGT (IU/L)	39.00 (25.00–72.00)	47.50 (28.00–82.75)	37.00 (30.75–53.75)	0.724

RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; PLT, platelet; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; GLB, globulin; crea, creatinine; TG, triglycerides; CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALP, alkaline phosphatase; GGT, gamma glutamyl-transferase.

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