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Genetic Variations Associated with Anti-Tuberculosis Drug-Induced Liver Injury

Yifan Bao¹, Xiaochao Ma², Theodore P. Rasmussen¹, and Xiao-bo Zhong¹

¹Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, 69 North Eagleville Road, Storrs, CT 06269, USA

²Center for Pharmacogenetics, Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261, USA

Abstract

Purpose of this Review—In order to combat the development of drug resistance, the clinical treatment of tuberculosis requires the combined use of several anti-tuberculosis (anti-TB) drugs, including isoniazid and rifampicin. Combinational treatment approaches are suggested by the World Health Organization (WHO) and are widely accepted throughout the world. Unfortunately, a major side effect of the treatment is the development of anti-tuberculosis drug-induced liver injury (AT-DILI). Many factors contribute to isoniazid- and rifampicin-mediated AT-DILI and genetic variations are among the most common factors. The purpose of this review is to provide information on genetic variations associated with isoniazid- and rifampicin-mediated AT-DILI.

Recent Findings—The genetic variations associated with AT-DILI have been identified in the genomic regions within or near genes encoding proteins in the following pathways: drug metabolizing enzymes (*NAT2*, *CYP2E1*, and *GSTs*), accumulation of bile acids, lipids, and heme metabolites (*CYP7A1*, *BSEP*, *UGTs*, and *PXR*), immune adaptation (*HLAs* and *TNF- α*), and oxidant challenge (*TXNRD1*, *SOD1*, *BACH1*, and *MAFK*).

Summary—The information summarized in this review considers the genetic bases of risk factors contributing to AT-DILI and provides information that may help for future studies. Some of the implicated genetic variations can be used in the design of genetic tests and serve as biomarkers for the prediction of isoniazid- and rifampicin-mediated AT-DILI risk in personalized medicine.

Keywords

Anti-tuberculosis drugs; Drug-induced liver injury; Genetic variations; Isoniazid; Rifampicin

Correspondence to: Xiao-bo Zhong.

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Introduction

Tuberculosis (TB) is a global health problem, which is caused by infection with bacterium *Mycobacterium tuberculosis*. Though the disease can affect the whole body, the lungs are the most significantly affected organs. In 2015, 10.4 million people were infected with TB and 1.4 million people died of TB based on the “Global Tuberculosis Report 2016” from the WHO. A much higher infection rate (16–27 times greater) of developing TB exists in people living with human immunodeficiency virus (HIV) as compared to those without HIV infection based on the WHO report. Approximately 60% of new TB cases occurred in six countries in Asia and Africa, including India, Pakistan, Indonesia, China, Nigeria, and South Africa. TB has been well controlled in the USA with an incidence rate of 2.9 cases per 100,000 persons in 2016 based on the Centers for Disease Control and Prevention of the USA. The financial cost for treatment of TB is \$6.6 billion (US dollars) annually around the world. To restrict the spread of TB and assist infected people, treatment with anti-tuberculosis (anti-TB) drugs is required.

Current anti-TB drug therapy is effective, but accompanied by a high risk of severe adverse drug reactions, particularly anti-TB drug-induced liver injury (AT-DILI). Throughout the years, a dozen anti-TB drugs have been discovered and developed, which can be classified into five groups according to the clinical usage [1, 2]. Group 1 consists of first-line core drugs (including isoniazid, rifampicin, pyrazinamide, and ethambutol) while groups 2–5 include second-line drugs, including fluoroquinolones, bacteriostatic drugs, and others with limited utility [2]. The usage of anti-TB drugs accelerates the development of multidrug-resistant (MDR) *Mycobacterium tuberculosis* through a selection process. Anti-TB drugs kill bacteria that lack drug resistance and leave those that survive the treatment to proliferate. The proliferation of MDR TB leads to relapse and no single drug can treat them effectively. In 2014, 480,000 out of the 9.6 million people with TB infection were reported to have resistant TB variants [3]. In response to this challenge, clinical treatment standards suggested by the WHO require the use of a combination of anti-TB drugs to effectively control relapse [4]. The treatment usually consists of an initial phase that kills *Mycobacterium tuberculosis* and a continuous phase that prevents relapse. Typically, the initial phase consists of a 2-month co-treatment with isoniazid, rifampicin, pyrazinamide, and ethambutol. The continuous phase consists of a subsequent 6-month administration of both isoniazid and rifampicin. Therefore, to achieve a full treatment course, a total of 8 months of co-treatment with isoniazid and rifampicin is needed. Isoniazid is activated by a bacterial catalase-peroxidase enzyme (katG) secreted by *Mycobacterium tuberculosis*. The activated compound spontaneously conjugates to reduced nicotinamide adenine dinucleotide (NADH), which blocks the formation of mycolic acid, a required component of the mycobacterial cell wall [5]. The mechanism of rifampicin in inhibition of tuberculosis is to arrest DNA-directed RNA synthesis within *Mycobacterium tuberculosis* through interaction with the β subunit of the bacterial RNA polymerase [6].

Co-treatment with isoniazid and rifampicin can result in severe adverse effects, the most common being AT-DILI, which has been known for a long time [7–9]. AT-DILI is often diagnosed by the occurrence of elevated levels of liver function biomarkers, including aspartate aminotransferase (AST), alanine transaminase (ALT), and total bilirubin levels

[10]. Hepatotoxicity induced by anti-TB drugs can lead to irreversible liver failure that requires liver transplantation [11]. If not recognized, such hepatotoxicity can be fatal. Though children are believed to have a lower risk of drug-induced liver injury in general, one study indicates that they have a higher risk of AT-DILI after co-treatment with isoniazid and rifampicin (6.9% in children vs. 2.7% in adults) [12]. Failure of the TB treatment itself can also be a consequence of drug-induced liver injury. Unfortunately, AT-DILI is difficult to predict. Many factors contribute to the variability of AT-DILI, including genetic (genetic variations), physiological (age and gender), pathological (liver diseases), and environmental factors (dietary compounds). The influence on AT-DILI by non-genetic risk factors is summarized in Table 1. Increasing age is a risk factor for AT-DILI [13]. Females have an increased risk [14]. In addition, people with hepatitis C virus (HCV) infection have an increased risk [15, 16]. Some compounds in fruits and food supplements can reduce the risk of AT-DILI due to their abilities to inhibit CYP2E1 activity [17] or to increase anti-oxidative activities [18–20].

Genetic variations associated with AT-DILI have been extensively studied. With the advantage of genome-wide association studies (GWAS), genetic variants have been identified that are associated with the AT-DILI. Previous reviews have discussed mechanisms and genetic factors that affect isoniazid-induced liver injury [21–23]. However, in a clinical setting, co-treatment with both isoniazid and rifampicin is much more common. This review sheds a light on recent discoveries concerning the contribution of genetic variants to AT-DILI caused by co-treatment with isoniazid and rifampicin. Important pathways and mechanisms of AT-DILI and associated genetic variations will be discussed and supported by clinical data and animal research.

General Molecular Mechanisms of Drug-Induced Liver Injury

A three-step working model of drug-induced liver injury (DILI) has been developed by Russmann et al. to illustrate general molecular mechanisms of DILI [24]. In the first step, the parent drug (or more frequently its reactive metabolites) initiates liver injury through impacts on cell stress, inhibition of mitochondrial function, or modulation of immune reactions. Genetic variations in genes encoding drug-metabolizing enzymes (phase I and II) and transporters may be associated with alterations of drug metabolism and an increased risk for the development of DILI. Genetic variations in genes encoding proteins involved in cell stress pathways, such as accumulations of lipids and bile acids, may also have an impact on an increased risk of DILI. In the second step, initial injury may lead to mitochondrial permeability transition (MPT) caused via the intrinsic or extrinsic pathway. The intrinsic pathway involves activation of intra-cellular stressor cascades and proapoptotic proteins. The extrinsic pathway is activated by immune reactions, sensitized to TNF- α , and modulated by cytokines. Genetic variations in genes encoding TNF- α , cytokines, and other immune proteins may have an impact on an increased risk of development of DILI. In the third step, the mitochondrial permeability transition leads to hepatocyte death via apoptosis or necrosis. Increased oxidant challenge may increase mitochondrial permeability transition and hepatocyte death. Genetic variations in genes encoding proteins involved in the alteration of oxidant challenge may have an impact on an increased risk of development of DILI.

Specific Molecular Mechanisms of Isoniazid/Rifampicin-Induced Liver Injury

Metabolism of Isoniazid and Its Metabolites by Phase I and II Enzymes

When entering the liver, isoniazid can be acetylated to form acetyl-isoniazid by *N*-acetyltransferase 2 (NAT2) or hydrolyzed to form isonicotinic acid and hydrazine. Acetyl-isoniazid can be further metabolized to acetyl hydrazine. Metabolomic analysis also indicates the presence of other isoniazid metabolites and hydrazones in human urine samples [25]. Both acetyl hydrazine and hydrazine can be metabolized by CYP2E1 to form active intermediate products. These products have much higher activities causing cytotoxicity than isoniazid itself and are able to covalently bind to liver macro-molecules, leading to mitochondrial injury [22, 26]. Acetyl hydrazine is further detoxified through conjugation by glutathione *S*-transferases (GSTs). Therefore, alterations on enzymatic functions of NAT2, CYP2E1, and GSTs in isoniazid metabolism may contribute to AT-DILI.

Accumulation of Bile Acids, Lipids, and Heme Metabolites Caused by Co-treatment with Isoniazid and Rifampicin

One cause of cell stress is the accumulation of bile acids in hepatocytes. Bile plugs are found in humanized PXR mice treated with isoniazid and rifampicin [27]. Another study also found that bile acid accumulation promotes AT-DILI [28]. Down-regulated expression of bile acid transporters, bile salt export pump (BSEP), and sodium taurocholate co-transporting polypeptide (NTCP) are found in mice co-treated with isoniazid and rifampicin, which is associated with an increased risk of the AT-DILI [28]. Increased expression of CYP7A1 occurs in Wistar rats co-treated with isoniazid and rifampicin, which results in excess bile acids based on histo-pathological studies [29]. Therefore, alterations of CYP7A1 and BSEP in the accumulation of bile acids may contribute to AT-DILI.

Accumulation of lipids results in another cause of cell stress. Rifampicin can cause liver toxicity by its effect on increasing lipid accumulation. Lipid accumulation is upregulated by PPAR γ activation. Treatment with rifampicin (200 mg/kg) in mice for 4 weeks results in elevated hepatic lipids caused by induction of PPAR γ through rifampicin-mediated activation of pregnane X receptor (PXR) [30]. A recent toxico-proteomics study suggests that the PPAR γ signaling pathway is involved in rifampicin-induced liver injury [31]. In the study, mice were treated with either a low dose (177 mg/kg) or a high dose (422.5 mg/kg) of rifampicin. PPAR γ and five downstream proteins, including apolipoprotein C-III, acyl-CoA-binding protein, 3-ketoacyl-CoA, thiolates A and B, and perilipin-2, were down-regulated in the mice treated with rifampicin. As an important receptor in lipid metabolism, the increase of PPAR γ levels indicates a potential mechanism for rifampicin-induced liver injury. The study also identified that cytochrome P450, glutathione metabolism, chemical carcinogenesis, and related proteins increased in a dose-dependent manner in rifampicin-treated mouse livers [31]. Therefore, alterations of transcriptional regulatory functions of PXR and PPAR γ may contribute to rifampicin-induced liver injury.

PXR plays an important role in the regulation of heme biosynthesis. The initiation of cell stress can be triggered by the accumulation of a heme biosynthesis intermediate, pro-

toporphyrin IX (PPIX) in the liver. PPIX accumulation is associated with hepatotoxic effects mediated by the blocking of bile acid efflux [32, 33]. The liver is responsible for the biosynthesis of 15% of heme in the body [34]. An increased risk of AT-DILI after co-treatment with isoniazid and rifampicin is associated with the accumulation of PPIX in the liver, which is regulated by PXR in the heme biosynthesis pathway [27]. Other factors involved in PPIX accumulation are also involved in liver injury caused by isoniazid and rifampicin co-therapy. Ferrochelatase expression is reduced in human hepatocytes and mouse livers, while aminolevulinic acid synthase 1 (ALAS1) and breast cancer resistance protein are upregulated after AT-DILI [35, 36]. Since PXR is known to upregulate ALAS1 expression in the liver, the co-treatment might cause PPIX accumulation by activating PXR and other factors involved in PPIX metabolism. Therefore, alteration of the regulatory function of PXR and the accumulation of PPIX may contribute to AT-DILI.

Immune-Mediated Toxicity Caused by Co-treatment with Isoniazid and Rifampicin

Isoniazid can induce liver injury through activation of the immune system. A recent study found anti-isoniazid antibodies in the serum of patients with liver failure induced by isoniazid, while these antibodies were not present in the serum of patients who did not develop liver injury [37]. Patients with isoniazid-induced liver injury carried isoniazid-specific CD4⁺ T cells, suggesting that adaptive immune responses can be involved in the liver injury process [38]. The immune response in isoniazid-induced liver injury is triggered by proteins that are covalently modified by isoniazid metabolites [39]. When mice were injected with isonicotinic acid-modified proteins, the number of immune cells expressing a series of immune activation markers, including CD11b, CD8, CD4, CD45R, and KI67, were increased [39].

Problematic immune system adaptation can occur in subjects co-treated with isoniazid and rifampicin. T lymphocytes were used to detect the immunological effects in vitro. Once activated in vivo, T cells can be isolated and are able to respond after challenge to the same immunogenic reagents in vitro. Peripheral blood mononuclear cells (PBMC) from patients who developed AT-DILI during co-treatment with isoniazid and rifampicin contain drug-responsive T lymphocyte cells which can be grown in vitro [38]. CD4⁺ clones were found in all patients who had AT-DILI. Activation of the clones with isoniazid was HLA-restricted and dependent on antigen-presenting cells. *HLA* genotypes may be important for the susceptibility to AT-DILI. Tumor necrosis factor- α (TNF- α), an inflammatory cytokine known to play an important role in the pathogenesis of drug-induced liver injury, was also significantly increased in livers from mice co-treated with isoniazid and rifampicin [40].

Increased Oxidant Challenge Caused by Co-treatment with Isoniazid and Rifampicin

Oxidant challenge is a factor in hepatotoxicity induced by co-treatment with isoniazid and rifampicin. Glutathione, a mitigator of oxidant challenge, was found to be depleted in mitochondrial fractions of mouse liver after isoniazid and rifampicin co-treatment [41]. In addition, this study found increased levels of glutathione disulfide bonds, which further supports that oxidative damage occurs in the liver after co-treatment [41]. An antioxidant protein, metallothionein, has been shown to have protective effects. When metallothionein is knocked down in mice, significant elevations of ALT and AST are observed [41].

Glutathione *S*-transferases (GSTs) may play an important role in the alteration of oxidant challenge in co-treatment with isoniazid and rifampicin.

Enzymes involved in the oxidant challenge may be important in susceptibility for AT-DILI. Thioredoxin reductase 1 (TXNRD1), encoded by the *TXNRD1* gene, is an enzyme with a broad substrate specificity related to oxidant challenge [42]. TXNRD1 regulates cell growth and protects cells against oxidative damage. Deficiency of TXNRD1 enhances cytotoxicity in cancer cells via a thioredoxin-independent mechanism [43]. The antioxidant enzyme superoxide dismutase 1 (SOD1) catalyzes a reaction that converts superoxide anions into molecular oxygen. Decreased function of SOD1 caused by genetic polymorphisms promotes accumulation of toxic super-oxide radicals [44]. Mice with a deficiency of *SOD1* show elevated levels of oxidative damage in various tissues [45]. Other important proteins involved in antioxidant defense processes include BTB and CNC homology 1 (BACH1) and MAF BZIP transcription factor K (MAFK). Both BACH1 and MAFK are transcription factors that participate in NRF2-mediated antioxidant response in control of the redox signaling pathways [46].

In summary (Fig. 1), the proteins and pathways that can contribute to a higher risk of AT-DILI after co-treatment with isoniazid and rifampicin include the following: (1) drug metabolizing enzymes (NAT2, CYP2E1, and GSTs); (2) proteins in the regulation of accumulation of bile acids, lipids, and heme metabolites (CYP7A1, BSEP, UGTs, and PXR); (3) proteins involved in immune adaptation (HLAs and TNF- α); and (4) proteins involved in oxidant challenge (GSTs, TXNRD1, SOD1, BACH1, and MAFK).

Genetic Variation Associated with AT-DILI

Genetic variants and their potential mechanisms whereby they contribute to AT-DILI are discussed on a gene-by-gene basis with a focus on population-based association studies and molecular mechanisms as defined in animal models.

NAT2—NAT2 is a phase II enzyme responsible for acetylation of xenobiotics. The activity of NAT2 is impacted by genetic polymorphisms. Based on their endogenous enzyme activities conducted by NAT2, patients can be classified into rapid acetylators (RA), intermediate acetylators (IA), and slow acetylators (SA). Correlation of NAT enzyme activity with alleles of *NAT2* has been conducted [47]. *Nat2**4 is the normally active wild-type allele. Most variant alleles differ from *Nat2**4 by one or more single-nucleotide polymorphisms with *5, *6, *7, and *14 alleles responsible for SA phenotypes.

Clinical studies have revealed the correlation between the *NAT2* genetic variations and AT-DILI. In a meta-analysis involving 38 studies with 2225 patients and 4906 controls [48], SA alleles of the *NAT2* gene were significantly associated with a higher risk of AT-DILI. Additional clinical studies support the association between SA phenotypes and an increased risk of AT-DILI in Thai [49], Indonesian [50], and Singapore populations [51]. Another study conducted in Moroccan patients observed no NAT2 RA (*Nat2**4) alleles in the patients that developed AT-DILI [52]. Based on these findings, a model for predicting risk of AT-DILI based on *NAT2* genotypes was established for Japanese patients [53]. Using clinical

data from 366 Japanese patients with TB, the model increases the effectiveness of the clinical prediction of AT-DILI from less than 33% to over 93.2%.

The molecular mechanisms whereby *NAT2* polymorphisms contribute to AT-DILI are not clear. Studies using animal models may provide insights into the molecular mechanisms of AT-DILI involving slow NAT metabolizers. In an animal study, *NAT2* deficiency did not change the covalent binding of isoniazid to host tissues during liver injury [39]. Using a high-dose treatment of isoniazid in an animal model, an overdose of isoniazid resulted in the accumulation of oleoyl-I-carnitine and linoleoyl-I-carnitine in the liver, which further led to mitochondrial dysfunction [54]. A recent study used *Nat1/2^{-/-}* mice as a genetic model for NAT slow metabolizers revealed increases in interactions of isoniazid with certain endobiotics, including fatty acids and vitamin B6, which may provide novel insights into the mechanisms of isoniazid-induced liver injury [55]. However, more experiments are required to illustrate the role of *NAT2* variants in a higher risk of AT-DILI.

In summary, genetic variants of *NAT2* can serve as predictors for a higher risk of AT-DILI. The FDA has implemented an annotation label for isoniazid/rifampicin/pyrazinamide and *NAT2*, which states: “The rate of acetylation does not significantly alter the effectiveness of isoniazid. However, slow acetylation may lead to higher blood levels of the drug, and thus, an increase in toxic reactions.” Pharmacogenetic associations of the important *NAT2* variants with isoniazid-induced liver injury have been summarized in the PHarmGKB database [47], which can serve as a clinical guide for predicting the risk of development of AT-DILI in patients who carry *NAT2* genetic variants.

CYP2E1—CYP2E1 is a phase I cytochrome P450 enzyme that participates in drug metabolism. In anti-TB treatment with isoniazid, CYP2E1 is required to oxidize acetyl hydrazine to form an activated toxicant. The *CYP2E1* gene has several functional alleles summarized in CYP allele nomenclature [56]. These alleles have impacts on the CYP2E1 drug-metabolizing function. Among the alleles, two variants can be detected by *PstI*, *RsaI*, and *DraI* restriction enzyme digests. The alleles for the site detected by *PstI* and *RsaI* digests were designated as C1 and C2 alleles with C1 as the wild-type version leading to faster metabolism. The allele detected by *DraI* digests is designated as *D/C* alleles.

Associations between the *CYP2E1* alleles and AT-DILI have been extensively examined in various populations, but some of the results are contradictory. A meta-analysis combining the data from eight papers concludes that the *CYP2E1 C1/C1* genotype is associated with a higher enzyme activity and a higher risk for AT-DILI [57]. The studies included in the meta-analysis examined Chinese, Korean, and Japanese populations. Recently, an updated meta-analysis assessed a larger set of data from 26 studies with a total of 7423 participants [58]. The results confirmed an increased risk of the *CYP2E1 C1/C1* genotype for occurrence of AT-DILI. However, no effect of the *DraI* polymorphisms associated with decreased CYP2E1 activity was found for AT-DILI risk.

Molecular studies provide evidence for an important role of CYP2E1 in AT-DILI. CYP2E1 inhibitors have proven to have a protective effect during co-treatment with isoniazid and rifampicin [59]. Mice treated with a CYP2E1 inhibitor, kaempferol, before co-treatment

with isoniazid and rifampicin have a milder liver injury upon histological examination, such as hepatocellular disintegration and vacillation. The study controls for other factors, such as plasma concentrations of isoniazid and rifampicin, and levels of other critical enzymes in AT-DILI, such as NAT2 and CYP2E1. Another CYP2E1 inhibitor, mannitol, has also proven to have a preventative effect on isoniazid- and rifampicin-mediated AT-DILI in mouse liver [60]. The effect of this CYP2E1 inhibitor shows that the CYP2E1 activity is essential for the development of AT-DILI. Furthermore, using *Cyp2e1*-null mice to completely remove CYP2E1 activity, a recent study finds a role for CYP2E1 for the development of elevated levels of serum cholesterol, triglycerides, and hepatic bile acids after isoniazid treatment [61]. This study concludes that accumulation of isoniazid metabolites mediated by CYP2E1 may play a role in isoniazid-induced DILI through the enhancement of bile acid accumulation and mitochondria β -oxidation. In conclusion, genetic variants of the *CYP2E1* gene can serve as a predictor for the risk of developing AT-DILI.

In addition to CYP2E1, association of genetic variations in several other drug-metabolizing CYP enzyme genes with the occurrence of AT-DILI was examined in a Chinese Han population consisting of 89 AT-DILI patients and 356 control patients [62]. The genetic variants assessed included one SNP in *CYP3A4* (rs12333983), two in *CYP2C19* (rs11568732 and rs4986894), and two in *CYP2C9* (rs4918758 and rs9332098). However, this study concluded that no association of these SNPs correlated with development of AT-DILI in the Chinese population. A study with *Cyp3a*-null mice also concludes that CYP3A enzymes have no effect on the systemic pharmacokinetics of isoniazid in mice and are not involved in bioactivation of isoniazid and development of AT-DILI [63].

GSTs—Glutathione *S*-transferases (GSTs) are important phase II drug-metabolizing enzymes that provide protection from oxidative stress. They are responsible for conjugating glutathione to substrates, including xenobiotics and reactive oxygen species (ROS) generated during liver injury. Therefore, they protect macromolecules from the attack of oxidative intermediates induced by isoniazid and rifampicin.

Absence of GST activity renders the liver susceptible to AT-DILI. The two major GST members involved in the conjugation of isoniazid are GSTM1 and GSTT1. Absence of GST activities is caused by homozygous null mutations at these two genomic loci. As a result, the lack of protection from oxidants can cause liver injury. A clinical study in an Indian patient population indicated that *GSTM1* and *GSTT1* null genotypes are associated with high rates of AT-DILI [64]. A meta-analysis that considered 451 relevant studies before 2013 found a strong association between certain *GSTM1* genotypes and an elevated risk of AT-DILI after combined treatment with isoniazid and rifampicin [65]. Association of *GSTT1* null genotypes with the risk of AT-DILI only exists in some East Asian populations [65]. Another meta-analysis-based study of 20 published association reports collectively conducted on over 5000 subjects also revealed the presence of a significant association between *GSTM1* genotypes and risk of AT-DILI, but no association with *GSTT1* genotypes [66]. Recently, another GST member, GSTP1, was found to associate with AT-DILI. A tag-SNP of the *GSTP1* gene has a significantly different frequency in an AT-DILI group as compared to a non-AT-DILI group [67]. DNA methylation levels in a CpG island in the promoter region of the *GSTP1* gene also associate with AT-DILI [68].

The role of the genetic variations in the *GST* genes at risk for AT-DILI will require additional studies to determine their roles in the detoxification of isoniazid and rifampicin intermediates, which may be mechanistically involved in oxidative damage.

CYP7A1—CYP7A1 is the enzyme responsible for converting cholesterol to 7- α -hydroxycholesterol, which is the first and rate-limiting step in bile acid synthesis [69]. Genetic variants of the *CYP7A1* gene have been studied in human populations [70]. *CYP7A1* genetic variants have been associated with defects in cholesterol and bile acid homeostasis [71]. Some of the variants are associated with higher susceptibility to cholesterol or bile acid-related human diseases, including gallbladder stone disease [72, 73], coronary artery disease [74], and biliary cirrhosis [75]. A SNP (rs3808607) in the *CYP7A1* gene is also associated with a higher susceptibility to tuberculosis infection in a Moroccan population [76]. Certain haplotypes in the *CYP7A1* gene region have shown a higher risk of AT-DILI in a Chinese patient population [77].

The molecular mechanisms whereby CYP7A1 participates in AT-DILI have been investigated. Using Wistar rats as a model, one study showed a crucial role of CYP7A1 in anti-TB drug-induced hepatotoxicity [29]. In the animal group with AT-DILI, expression of CYP7A1 is significantly elevated in comparison to the control animal group without AT-DILI. An excess of bile acids was accompanied by elevated expression of CYP7A1 and damage of hepatocytes, which was confirmed by histopathological methods.

Although the association of genetic variations in *CYP7A1* with AT-DILI has been documented in a Chinese population [77], more population-based studies are needed to establish a solid correlation between the genetic variants of the *CYP7A1* gene and AT-DILI.

BSEP—The bile salt export pump (BSEP) is an efflux transporter, critically involved in the secretion of bile salts into the bile produced by the liver [78]. Bile acid secretion can be impaired or abolished by genetic variants in the *ABCB11* gene, which encodes the BSEP protein. The impact of genetic polymorphisms in *ABCB11* is associated with AT-DILI in a Chinese cohort consisting of 89 AT-DILI patients and 356 matched control patients [79]. The study suggests that genetic variants of the *ABCB11* gene might contribute to AT-DILI, but larger and more varied populations are required to further confirm the findings.

UGTs—UDP glucuronosyltransferases (UGTs) are phase II drug-metabolizing enzymes that are responsible for conjugating bilirubin with glucuronic acid, a product of heme catabolism. Among the 22 UGT isoforms in humans, UGT1A and 2B play essential roles in the metabolism of bilirubin in the liver. Association of a subset of *UGT* polymorphisms with AT-DILI has been described. One study composed of 17 AT-DILI and 71 control patients found a positive association between *UGT1A1**27 and *UGT1A1**28 (two alleles causing reduced expression of UGT1A1) and an increased risk of AT-DILI [80]. However, other SNPs (rs4148323 and rs8330) in the *UGT1A1* gene have no significant correlation with AT-DILI in a study that assessed a Chinese Han population containing 4488 patients [77]. In addition, a recent study examined the relationship between *UGT2B7* SNPs and AT-DILI in a Chinese Han population [81]. This study found no association between SNPs in *UGT2B7* and AT-DILI.

PXR—Genetic polymorphisms in the pregnane X receptor (PXR) gene have been examined in several studies as potential risk factors for AT-DILI [82–84]. A study that examined genotypes and haplotypes with six SNPs in the *PXR* gene in female Chinese populations found associations between *PXR* genetic polymorphisms (rs2461823 and rs7643645) and increased risk of AT-DILI [82]. Genotypes of another *PXR* polymorphism (rs3814055) were found to associate with a greater risk of AT-DILI in a study of 106 Indonesian patients [83]. SNPs rs2461823 and rs7643645 are located in the first intron of the *PXR* gene. rs7643645 is located in an element that recruits hepatocyte nuclear factor 4 alpha (HNF4α). This SNP consists of an A to G transition and leads to a lowered binding affinity of HNF4α [85]. This finding suggests that the function of this SNP may have an impact on AT-DILI through repression of PXR via reduced HNF4α recruitment. Although rs7643645 is not in a similar cis-acting site for factor binding, it is in linkage disequilibrium with other SNPs located within sites to which hepatocyte nuclear factor 3-β may bind, again suggesting that transcriptional regulation of *PXR* may be involved in AT-DILI.

HLAs—Human leukocyte antigens (HLAs) are a complex of genes encoding major histocompatibility complex (MHC) proteins. *HLA* genes are classified into MHC class I, II, and III. MHC class I genes include *HLA-A*, *HLA-B*, and *HLA-C*, while MHC class II genes contain *HLA-DQA1/B1* and *HLA-DRA1/B1*. MHC class II proteins are primarily responsible for presentation of antigens whose origin is extracellular.

With normal MHC genes, drug-protein conjugates are not presented and do not trigger a Tcell response. However, in the context of certain mutations in *HLA* genes, drug-protein conjugation occurs and the conjugates are presented on the surface of antigen-presenting cells, which may activate T cell responses leading to hepatocyte damage of immune origin. Treatment of drug-specific T cells isolated from patients suffering from adverse effects brought on by co-treatment with rifampicin and isoniazid with MHC I and II neutralizing antibodies block the activation of Tcells, suggesting an influential role of HLA patient-specific genotypes in AT-DILI [86].

Several studies have examined associations of genetic polymorphisms in *HLA* genes with risk of AT-DILI. A case-control study explored the possible association of genetic polymorphisms in the *HLA-DQB1* gene with AT-DILI [87]. The frequency of the genotype *HLA-DQB1**05/*05 was significantly higher in AT-DILI patients as compared to control subjects in a multivariate analysis using the conditional logistic regression model. A higher allele frequency of *HLA-DQA1**03:02, *HLA-DRB1**08:01, or *HLA-DQB1**08:03 was also found in AT-DILI patients than in control subjects in a Kazakhstan population [88]. Further studies are needed to assess the association of *HLA* genotypes with a higher risk for developing AT-DILI in cohorts of patients in other populations.

TNF-α—Tumor necrosis factor-α (TNF-α), an inflammatory cytokine produced by activated immune cells, has been suggested to play an important role in drug-induced immune responses. A study has tested the hypothesis that genetic polymorphisms in the *TNF-α* gene are associated with AT-DILI [89]. The study compared the frequencies of the *TNF-α* genetic polymorphism – 308G/A in 77 patients with AT-DILI and 229 tolerant patients in a Korean population. The *TNF-α* genetic polymorphism – 308G/A is a G/A

transition at – 308 bps upstream of the transcription start site. The results revealed that – 308G/A was significantly associated with AT-DILI. The A allele is significantly linked with the higher TNF- α levels and a more severe outcome in immune diseases [90]. More studies are needed to confirm the association of *TNF- α* genetic polymorphisms with AT-DILI and to assess the potential role of inflammation as an AT-DILI mechanism.

TXNRD1—Thioredoxin reductase 1 (TXNRD1) is a key enzyme in response to oxidant challenge. It is responsible for reducing thioredoxin and is considered as a major contributor to selenium metabolism, which is involved in protecting cells against oxidative damage. A haplotype consisting of several SNPs in the *TXNRD1* gene is associated with the development of drug-induced liver injury, including injury caused by anti-tuberculosis drugs, antibiotics, and antiepileptic drugs in a Korean population [91]. Another study conducted in a Chinese population also found association between a haplotype involving the *TXNRD1* gene and increased susceptibility to AT-DILI [42]. Further studies are required to verify the association.

SOD1—Genetic polymorphisms in genes encoding key anti-oxidant enzymes, superoxide dismutases (SODs), are also associated with the susceptibility to AT-DILI. SODs convert superoxides into oxygen and hydrogen peroxide. There are three forms of SOD: cytoplasmic superoxide dismutase (SOD1), mitochondrial superoxide dismutase (SOD2), and extracellular superoxide dismutase (SOD3). Four SNPs in the three SOD genes were tested in 84 Korean patients suffering AT-DILI. The results indicate that SNP rs2070424 in the *SOD1* gene is significantly associated with AT-DILI and that this genetic variant may be a risk factor for AT-DILI in individuals from Korea [92].

BACH1 and MAFK—Association of genetic polymorphisms in several additional genes involved in antioxidant pathways, including nitric oxide synthase 2 (NOS2A), BTB domain and CNC homolog 1 (BACH1), and MAF bZIP transcription factor K (MAFK), with AT-DILI has been examined in a Japanese population [93]. The study concludes that a combination of *BACH1* and *MAFK* polymorphisms may serve as biomarkers to identify TB patients at high risk for the development of AT-DILI.

Conclusions

Tuberculosis (TB), caused by infection with bacterium *Mycobacterium tuberculosis*, is a global public health problem, affecting over 10 million people annually in the world. Co-treatment with isoniazid and rifampicin is effective to control TB and combats the spread and relapse of the disease. However, this combination therapy also can result in severe AT-DILI in susceptible individuals. Prediction of hepatotoxicity is critical in the treatment of TB and can guide the choice of safe medicines. Extensive efforts have been put in the recent decades to identify genetic polymorphisms and genes involved in the susceptibility to AT-DILI. Here, we have summarized updated findings in genetic polymorphisms and genes involved in drug-metabolizing enzymes, accumulation of bile acids, lipids, and heme metabolites, immune adaptation, and oxidant challenges and their association with a high risk of AT-DILI (Table 2 and Fig. 1).

Association of genetic polymorphisms in the *CYP2E1* and *NAT2* genes with AT-DILI has been clearly documented using large population-based association studies. The associations of “slow acetylation” phenotypes with higher blood levels of isoniazid and increased rates of toxic reactions have been incorporated into the FDA's drug label for the treatment of TB with isoniazid. The molecular basis of genetic polymorphisms in *CYP2E1* and *NAT2* genes with increased risk of AT-DILI has also been studied with cell-based assays and in animal models harboring genetic deficiencies.

Association of genetic polymorphisms in *GST* genes with higher susceptibility to AT-DILI has been supported by case-control patient population-based studies; however, potential mechanistic roles of genetic variations associated with AT-DILI have not been determined. Further studies are required to examine the molecular mechanisms of *GST* variants in detoxification of isoniazid and rifampicin.

Associations of genetic polymorphisms in other genes with higher susceptibility to AT-DILI have been documented in case-control patient population studies, including *CYP7A1*, *BSEP*, *UGT1A1*, *PXR*, *HLAs*, *TNF-α*, *TXNRD1*, and *SOD1*. However, these associations are supported by more limited studies with smaller sample sizes, often in highly specific patient populations. Further studies are required to confirm the associations using larger patient sample sizes across a broader spectrum of ethnic populations. Molecular mechanisms resulting in increased susceptibility to AT-DILI mediated by the genetic polymorphisms would benefit from additional research using in vitro or in vivo models. We can look forward to a better understanding of DILI mechanisms once such animal researches are more thoroughly explored.

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Fig. 1.

Proteins and pathways that contribute to an elevated risk of AT-DILI after co-treatment with isoniazid and rifampicin. CYP2E1: cyto-chrome P450 2E1; NAT2: N-acetyltransferase 2; GSTs: glutathione S-transferases; CYP7A1: cytochrome P450 7A1; BSEP: bile salt export pump; UGTs: UDP-glucuronosyltransferases; PXR: pregnane X receptor; HLAs: human leukocyte antigens; TNF- α : tumor necrosis factor alpha; TXNRD1: thioredoxin reductase 1; SOD1: superoxide dismutase 1; BACH1: BTB and CNC homology 1, basic leucine zipper transcription factor 1; and MAFK: MAF BZIP transcription factor K

Table 1
Non-genetic risk factors for AT-DILI

Risk factors		Impact on AT-DILI [references]
Physiologic	Age	Increased age is associated with increased risk of AT-DILI [13].
	Gender	Females have increased risk of AT-DILI [14].
Pathologic	Liver disease	HCV infection increases the risk of AT-DILI [15, 16]
Environmental	Diet (fruits and food supplements)	Certain compounds in fruits reduce the risk of AT-DILI [17–19].
		Food supplements (spirulina fusiformis) decrease the risk of AT-DILI [20].

Table 2
Genetic variations associated with AT-DILI in the co-treatment with isoniazid and rifampicin

Gene	Gene variations	Phenotype	Possible mechanism	References
<i>NAT2</i>	*2, *5, *6, *7	Slow acetylator	Lower efficiency of detoxifying the hepatotoxic agents	[30, 39, 48–52, 55]
<i>CYP2E1</i>	<i>C1/C1</i>	Rapid metabolizer	Higher induction of hepatotoxic agents	[57–63]
<i>GSTs</i>	<i>GSTM1 null</i> /homozygous	Slow metabolizer	Reduced efficiency of metabolism of hepatotoxic agents	[64–67, 82]
<i>CYP7A1</i>	Haplotype G-C or G-A of rs1457043-rs819287	Increased efficiency of bile acid synthesis	Increased toxicity due to increased accumulation of bile acids	[29, 76, 77]
<i>BSEP</i>	rs2287616	Unknown	Unknown	[70]
<i>UGT1A1</i>	*28	Inhibition of bilirubin glucuronidation and disruption of bile acid synthesis	Lead to heme accumulation	[77, 80, 81]
<i>PXR</i>	rs7643645 rs2461823	Increased expression of PXR	Higher lipid accumulation that triggers AT- DILI	[77]
<i>HLAs</i>	<i>HLA-DQA1*03:02 HLA-DQB*05</i>	Increased display of drug-conjugated peptides	Higher sensitivity of modified proteins	[87, 88]
<i>TNF-α</i>	-308G/A	Increased expression of TNF-α	More severe outcome in immune reactions	[90]
<i>TXNRD1</i>	Haplotype TCAGCC	Lower efficiency of reducing substrates involved in oxidative stress	Lower resistance to oxidative stress	[42, 91]
<i>SOD1</i>	rs2070424	Decreased superoxide radical conversion	Lower resistance to oxidative stress	[92]
<i>BACH1</i>	rs2070401	Unknown	Not determined	[93]
<i>MAFK</i>	rs4720833	Unknown	Not determined	[85]