REVIEW

Pharmacogenomics of anti-TB drugs-related hepatotoxicity

Puspita Das Roy, Mousumi Majumder & Bidyut Roy†

†*Author for correspondence*
Human Genetics Unit, Biological Sciences Division, Indian Statistical Institute, 203 B. T. Road, Kolkata 700108, India
Tel.: +91 33 2575 3213; Fax: +91 33 2577 3049; E-mail: broy@isical.ac.in

Anti-TB drug (ATD)-related hepatotoxicity is a worldwide serious medical problem among TB patients. Apart from acting on the bacteria, isoniazid, the principal ATD, is also metabolized by human enzymes to generate toxic chemicals that might cause hepatotoxicity. It has been proposed that the production and elimination of the toxic metabolites depends on the activities of several enzymes, such as *N*-acetyl transferase 2 (NAT2), cytochrome P450 oxidase (CYP2E1) and glutathione *S*-transferase (GSTM1). There is now evidence that DNA sequence variations or polymorphisms at these loci (*NAT2*, *CYP2E1* and *GSTM1*) could modulate the activities of these enzymes and, hence, the risk of hepatotoxicity. Since the prevalence of polymorphisms is different in worldwide populations, the risk of ATD hepatotoxicity varies in the populations. Thus, the knowledge of polymorphisms at these loci, prior to medication, may be useful in evaluating risk and controlling ATD hepatotoxicity.

Approximately a third of the world's population is latently infected with *Mycobacterium tuberculosis* with a 10% lifetime chance of developing TB. Approximately 9 million new cases of active tuberculosis are reported every year and, in 2004, an estimated 1.7 million people died of the disease. Currently, a combination of isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and/or ethambutol is commonly used against TB in different populations. Initially (in the 1950s), INH was thought to be safe without any side effects but, in early 1970s, several investigators documented large cases of INH-induced hepatotoxicity. The frequency of ill effects varies widely in different populations: approximately 1–30 in 100 individuals with INH and RMP treatment [1]. This hepatotoxicity is generally unpredictable and occurs in a small number of patients even when the drug has been given at the recommended doses. These adverse effects not only cause morbidity and mortality, but also lead to treatment interruptions and nonadherence, failure and relapse, which contribute to the continuing spread of the disease and the emergence of resistance to the TB drugs. Among the anti-TB drugs (ATDs), metabolism of INH, which is one of the frontline ATDs, has been studied extensively, and it has been proposed that acetylation by NAT2, oxidation by cytochrome P450 oxidase (CYP2E1) and detoxification by GST might play important roles in INH-induced hepatotoxicity. The metabolites generated by NAT2 are usually nontoxic. However, in some cases, CYP2E1-mediated reactions may lead to the formation of toxic reactive metabolites that are to be eliminated from the body by GST. Reactive metabolites can destroy hepatocytes either by interfering with cell homeostasis or by triggering immunologic reactions in which reactive metabolites, bound to hepatocyte plasma membrane proteins, may act as haptens [2].

Polymorphisms at different drug metabolic loci may contribute to interindividual differences in the pharmacological response to drugs. Pharmacogenetics and *N*-acetyltransferase are historically linked, and *in vivo* variation in NAT activity was one of the earliest recognized pharmacogenetic traits. The action of the NAT2 enzyme was first identified as the genetically controlled step responsible for the metabolism of INH [3]. In 1960, TB patients were routinely given INH and the incidence of peripheral neuropathy was observed in some patients. This was explained by unusually slow clearance of the toxic compound, acetyl hydrazine, from affected patients, and these individuals were termed as slow acetylators [4]. However, a few studies also reported that NAT2 fast acetylators are also susceptible to ATD hepatotoxicity [5,6]. Previously, NAT2 acetylation phenotypes were determined by an enzymatic method, and some earlier phenotypic studies have suggested an existence of such a predisposition although other studies failed to substantiate this observation [7–9]. Subsequently, individuals in an ethnic population were phenotyped as slow, intermediate or rapid acetylators on the basis of the acetylation capacity of the NAT2 enzyme.

Keywords: antituberculosis drugs, hepatotoxicity, isoniazid, pharmacogenomics

future medicine part of fsg

Most Caucasians and a minority of the Southeast Asians were observed to be slow acetylators [4]. Now it is proposed that not only the NAT2 enzyme, but also a few other enzymes (such as CYP2E1 and GSTM1) involved in ATD hepatotoxicity, display genetic polymorphisms, and the prevalence of polymorphic alleles varies widely in worldwide populations.

Different reports defined ATD hepatotoxicity in different ways, such as raised (2–3 times the upper limit of normal value) transaminases activity, clinical hepatitis, overt jaundice, withdrawal and rechallenge to drug. These different criteria to define hepatotoxicity resulted in different frequencies of hepatotoxicity in different studies. However, according to The International Consensus Meeting in Paris on drug-induced hepatic disorders, liver biochemical parameters more than two-times the upper limit of the normal value is regarded as hepatotoxicity [10]. In addition, approximately 10–20% of TB patients have asymptomatic increase in transaminase activities upon introduction of ATD that disappear after a few weeks.

In this review we discuss the reported risk factors for tuberculosis, the hepatotoxic effects of different ATDs, the possible mechanisms of toxicity and susceptible host genetic factors. Discussion will be emphasized on the published reports regarding how ATD hepatotoxicity has been explained by polymorphic alleles of host genetic factors in different populations.

Anti-TB drugs & toxicity

Frequency & risk factors

The overall frequency of clinically recognized hepatotoxicity affected by INH appears to be 1–30% in different populations, but there is striking age dependence. It is reported that the incidence of liver damage is rare in children aged under 20 years, 1–2% in the middle-aged group and 2–3% in those aged 50 years or more [11]. A high incidence of ATD hepatotoxicity has also been reported in patients with malnutrition [12], alcohol abuse [13] and carriers of hepatitis B and C viruses and HIV [14,15]. Most of the ATDs, for example, INH, RMP and PZA, are hepatotoxic. Like INH, PZA can also cause liver injury in 15% of the recipients, and jaundice might occur in 2–3% of these liver-damaged patients [16]. Like INH, it also shows dose-related adverse effects on the liver. Usually RMP is an agent of low toxicity, but it can interfere with the clearance of bilirubin and bile acids, thus causing gastrointestinal intolerance. In rare cases, it also causes hepatitis [17]. Ethambutol alone has side effects on vision, the level of serum uric acid, joint pain and so on. They may also have a synergistic effect on the liver function of the patients in combination with INH. The combinations of INH and RMP, and INH and PZA increase hepatotoxicity [18]. In another study, it was reported that the incidence of clinical hepatitis was 5–8% of patients treated with INH and RMP compared with 1% in those receiving only INH [19]. However, not all studies have suggested that combined treatment with RMP increases the incidence of INH-induced hepatotoxicity [16].

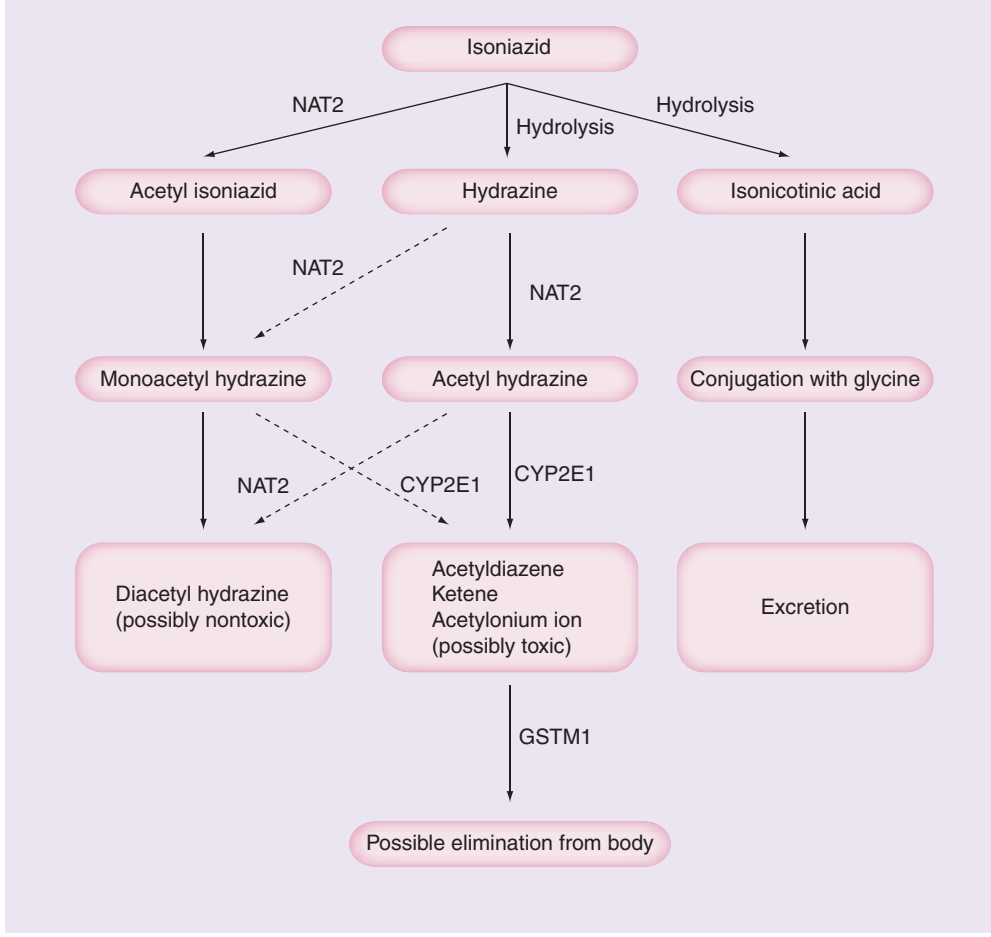
Clinical features & prevention

The first symptoms attributable to INH-induced hepatitis may appear within 4 weeks after starting treatment. Approximately 30% of clinical hepatotoxicity cases are observed within 2 months while two-thirds of the cases are noticed within 3 months of drug treatment [20]. Approximately 10% of the hepatotoxic patients suffer from jaundice alone. The remaining patients exhibit predominantly digestive complaints, such as anorexia, nausea, vomiting and abdominal pain. In most of the cases serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AP) and bilirubin are increased more than threefold the normal values, which also reflect malfunction of liver. In patients with clinical evidence of INH-induced hepatic injury, the overall mortality appears to be 10%. The factors most commonly associated with a fatal outcome include age greater than 50 years and continued use of the drug for several weeks after the onset of symptoms [21]. Both serum ALT and bilirubin, for example, markers of liver malfunction, return to normal or near normal values by the second/third week after discontinuation of the drug. The most important measure to prevent INH-induced liver injury is intensive monitoring of liver function of patients taking INH in combination with RMP or PZA. The importance of prompt discontinuation of treatment in patients suspected to have ATD-induced hepatitis, has been emphasized [22]. Since INH, RMP and PZA are the effective first-line drugs for tuberculosis, reintroduction of these drugs can be performed when liver function has returned to a normal level [23,24]. However, starting from a low dose of ATDs and closely monitoring liver function is mandatory under rechallenged conditions.

Possible mechanism of toxicity

Initially, acetylation of INH may result in formation of acetylisoniazid, which, in turn, may be hydrolyzed to acetyl hydrazine and, subsequently, possibly nontoxic diacetyl hydrazine by NAT2 [7]. Another hypothetical alternative pathway for synthesis of acetyl hydrazine is hydrolysis of INH to toxic hydrazine that may be acetylated to (possibly toxic) monoacetyl hydrazine. Although the mechanism is not clear, hydrazine has been shown to be hepatotoxic in animal study [22]. Nelson *et al.* [25] demonstrated that CYP2E1 mediated oxidation of monoacetyl hydrazine may generate hepatotoxins such as acetyldiazene, acetylonium ion, acetyl radical or ketene (Figure 1). These hepatotoxins could be detoxified by GSTs present in the liver. Alternatively, monoacetyl hydrazine may also be further acetylated by NAT2 to (possibly nontoxic) diacetyl hydrazine [26]. INH is also hydrolyzed by isoniazid hydrolase to isonicotinic acid that may be conjugated with glycine and excreted by the kidneys [22]. RMP is a potent inducer of CYP2E1, so it can increase the activity of this enzyme and thus regulate the production of hepatotoxic agents. This could be one of the possible mechanisms by which RMP enhances toxicity of INH. It is suggested that INH is converted to diacetyl hydrazine rapidly in rapid acetylators and excreted from the body, so rapid acetylators are less susceptible to ATD hepatotoxicity [27]. However, owing to the slow process of acetylation, less monoacetyl hydrazine is converted into diacetyl hydrazine in slow acetylators and most of the monoacetyl hydrazine is oxidized into toxic products by CYP2E1. So slow acetylators become susceptible to ATD hepatotoxicity if toxic products are not eliminated from the liver. In NAT2 slow acetylators, owing to hydrolysis, more INH may also be converted to toxic hydrazine, which may increase ATD hepatotoxicity.

Figure 1. Suggested metabolic pathway of isoniazid and metabolites by NAT2, CYP2E1 and GSTM1.



CYP: Cytochrome P450; GST: Glutathione-*S*-transferase; NAT2: *N*-acetyl transferase 2.

Polymorphisms & anti-TB drug hepatotoxicity

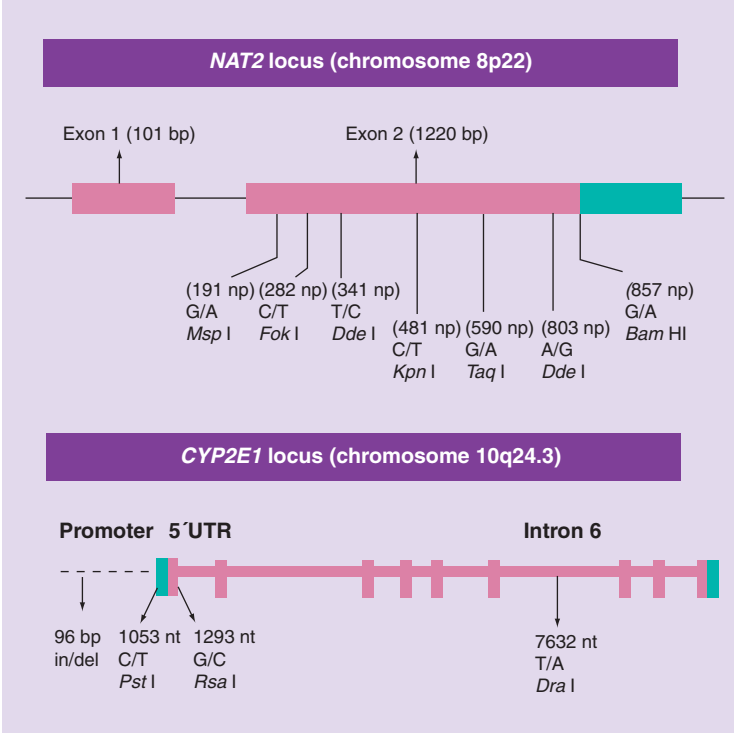
Several investigators have studied the association between polymorphisms at different drug-metabolizing (DM) loci and the risk of ATD hepatotoxicity in different populations. It was known for a long time that NAT2 slow acetylators are susceptible to INH hepatotoxicity. Now it is proposed that not only the NAT2 enzyme, but a few other enzymes (such as CYP2E1 and GSTM1) may also be involved in ATD hepatotoxicity. In the following sections, polymorphisms at these loci, as well as association between polymorphism and risk of ATD hepatotoxicity, published from different laboratories, will be discussed.

NAT2 *polymorphisms*

In humans, NAT2 reduces therapeutic concentrations of INH by *N*-acetylation. The acetylation derivative of INH exhibits at least 100-times less activity *in vivo* and 500-times less activity *in vitro* against *M. tuberculosis*. It has been observed that *N*-acetyl transferase of *M. tuberculosis* increases survival of the bacteria during INH treatment [28]. Therefore, acetylation of INH by bacterial NAT may render it inactive. The endogenous NAT of *M. tuberculosis* might have a distinct role in its growth, but it may change the sensitivity of the bacteria to INH [29]. In humans, NAT2 plays important roles in determining INH hepatotoxicity, and several polymorphic sites have been reported at *NAT2*. Therefore, it is essential to understand the correlation between genetic variations/polymorphisms at *NAT2* and acetylation capacity of the enzyme. The *NAT2* locus contains several SNPs but few of them are monomorphic in different ethnic populations. Now, it is obvious that genotyping at several SNPs, may be needed to determine the proper acetylation status of an individual in a population [101]. Since all the reported studies were based on case–control samples and approximately 50% of the individuals are heterozygous at two or more SNPs [30], a software is to be used to identify the pair of alleles or haplotypes present in an individual of a population [30,31]. This is the best possible estimation of a haplotype pair in an individual. At present, most of the investigators study seven or less SNPs at nucleotide positions (np) (191 [G/A], 282 [C/T], 341 [T>C], 481 [C>T], 590 [G>A], 803 [A>G] and 857 [G>A]) at exon 2 on *NAT2* locus to determine the acetylation status of individuals in different association studies (Figure 2). Alleles at a SNP could be determined by PCR-restriction fragment length polymorphism (RFLP) and instead of analyzing the genotype data at these polymorphic sites separately, genotypes are generally expressed as alleles/haplotypes, such as *NAT2*4*, *NAT2*5*, *NAT2*6*, *NAT2*7*, *NAT2*12* and so on, depending on the arrangements of nucleotides (G/A, C/T, T/C, C/T, G/A, A/G and G/A at 191, 282, 341, 481, 590, 803 and 857np respectively) at the above-mentioned polymorphic sites. Since most of the studies deal with unrelated case–control populations, a software (say PHASE v2.1.1, a statistical programme for haplotype reconstruction) is used to determine the haplotype pair in each individual from the genotype data [102]. Few of the SNPs (such as 341np T>C, 481np C>T and 803np A>G) are in strong linkage disequilibrium (Roy P *et al*. unpublished data, [32]), so a genotype at SNP 341np T>C could provide allelic information at another two SNPs (481np C>T and 803np A>G). Few SNPs are less frequent or monomorphic in different populations, such as 191np G/A in Indian population [32]. The common allele (i.e., *NAT2*4*) having common nucleotides at all polymorphic sites is known as the NAT2 rapid acetylating allele. Polymorphisms at 481 and 803np do not change the acetylation status, but variant alleles/haplotypes [101] having at least one variant nucleotide at any one of polymorphic sites at 341, 590 and 857np are known as slow acetylating alleles (e.g., NAT2*5, *NAT2*6B* and *NAT2*7A*). Therefore, individuals carrying two rapid acetylating alleles (such as the *NAT2*4/NAT2*4* genotype) in the pair of chromosomes are rapid acetylators; carrying two slow acetylating alleles (such as the *NAT2*5/NAT2*6B* genotype) are slow acetylators; and those carrying one slow and one rapid acetylating allele (i.e., the *NAT2*4/NAT2*5* genotype) are intermediate acetylators.

Using haplotype data, investigators reported that frequencies of NAT2 fast, intermediate and slow acetylators are similar in the Caucasian [33] and Indian population [30]. Frequencies of NAT2 fast, intermediate and slow acetylators are also similar in the Japanese and Chinese population [34]. Therefore, it could be noted that NAT2 slow acetylators are more frequent in Caucasian and Indian populations, whereas they are less frequent among Japanese and Chinese populations (Table 1). Studies on Japanese and Taiwanese populations used the PCR-RFLP genotyping method, at *KpnI* (481np

Figure 2. Polymorphic and corresponding restriction enzyme cutting sites at *NAT2* and *CYP2E1*.



Distance between two sites is not in proper scale.
CYP: Cytochrome P450; NAT2: *N*-acetyl transferase 2.

C>T), *TaqI* (590np G>A) and *BamHI* (857np G>A) polymorphic sites, to discover the acetylation status of the patients and controls, and suggested high risk of ATD hepatotoxicity, with a RR of 28.0 and a 95% CI of 26–30 and an OR of 3.66; 95% CI of 1.58–8.49, respectively, in NAT2 slow acetylators [35,36]. Recently, a study on a Korean population [37] also observed that NAT2 slow acetylators had a significant risk of ATD-induced hepatotoxicity (3.8-fold risk, p = 0.005). We genotyped at *MspI*, *KpnI* and *BamHI* polymorphic sites in the Indian population [38], and Vuilleumier *et al*. (2006) [39] genotyped at 191np, *KpnI*, *TaqI* and *BamHI* polymorphic sites in a mixed population of Caucasians, Hispanics, Africans, South Americans and Asians. However, these two studies could not show association between ATD hepatotoxicity and NAT2 acetylation status. All the above-mentioned studies considered that loss of the *KpnI* site at 481np would lead to slow acetylation. But it has been reported that loss of *KpnI* site at 481np does not change the acetylation status in the phenotypic assay [101]. Therefore, the above-mentioned observations should be validated by genotyping at least one more SNP, (say 341 T>C) even though C481T (*KpnI* site) polymorphism is highly linked with the T341C polymorphism in Indian population that leads to slow acetylation (Das Roy P *et al*. unpublished data, [32]). Therefore, it is important to know the status of linkage disequilibrium in a population between C481T and T341C, if polymorphism at 481np is to be used to determine the acetylation status of an individual. However, care should be taken to choose appropriate SNPs for genotyping and classify the individuals as slow, rapid or intermediate acetylators, otherwise overestimation of slow acetylators may be obtained. This might become a source of error in the association study. However, studies with more sample sizes from different populations and genotyping at appropriate polymorphic sites are of great importance in obtaining a clear understanding of the association between NAT2 acetylation status and ATD hepatotoxicity.

CYP2E1 *polymorphisms*

Human CYP, which represents a large multigene family with differing substrate specificity, is important in the oxidation reactions of several exogenous and indigenous chemicals into their ultimate reactive forms. CYP2E1, one of the CYP enzymes, may convert acetyl hydrazine into hepatotoxins, such as acetyldiazene, ketene, acetylonium ion and so on, which can influence ATD hepatotoxicity [40] (Figure 1). The INH or its metabolite hydrazine could induce the activity of CYP2E1 in rat [41], depending on the dose of INH in blood. However, INH could also inhibit CYP2E1 activity, and this inhibition is more pronounced in humans with variant genotype at *CYP2E1* [42]. Therefore, individuals with a common genotype at *CYP2E1* may possess higher CYP2E1 activity compared with individuals with variant genotype at *CYP2E1* when treated with INH. The higher activity of CYP2E1 may increase the synthesis of hepatotoxins and, hence, risk of hepatotoxicity.

The activity of CYP2E1 is also modulated by polymorphisms at several sites on the locus. Two polymorphisms upstream of the *CYP2E1* transcriptional start site are detectable by *PstI* and *RsaI* restriction enzymes and appear to be in complete linkage disequilibrium (Figure 2). Based upon the presence [+] or absence [-] of *RsaI* and *PstI* recognition sequences, respectively, the common haplotype (*RsaI* [+].*PstI* [-]), and one of the variant haplotypes, (*RsaI* [-].*PstI* [+]), are designated as the 'c1' and 'c2'

Table 1. Distribution of acetylation status, genotypes and alleles in different populations.

		Acetylation frequency (%)			
Locus	***Population***	***Fast/Rapid***	***Intermediate***	***Slow***	
NAT2	Caucasian	6	37	57	
	Indian	4	36	60	
	Japanese	48	46	6	
	Chinese	35	47	18	
		Genotype/allele frequency (%)			
		****1A/*1A***	****1A/*5***	****5/*5***	****6 allele***
CYP2E1	European–American	92	7	1	10
	African–American	98	2	0	-
	Taiwanese	56	32	12	25
	Japanese	-	26	-	33
	Hawaiians	-	16	-	15
	Indian	98	2	0	20

alleles, respectively. Presently, this wild c1 haplotype has been designated as *CYP2E1*1A*, and the variant c2 haplotype has been renamed as the *CYP2E1*5* allele [103]. The *PstI* and *RsaI* polymorphic sites are present in a putative HNF-1 binding site, and thus may play important roles in the regulation of *CYP2E1* transcription and subsequent protein expression [43]. The *DraI* polymorphism at intron 6 of *CYP2E1* leads to a variant *CYP2E1*6* allele (absence of *DraI* restriction site). Another polymorphism (96 bp insertion/deletion or *CYP2E1*1D/*1C* polymorphism) at the 5´-regulatory region of this gene could also regulate enzyme activity. Several studies have reported that the variant *CYP2E1*5*, **6* and **1D* alleles are associated with enhanced enzyme activity [44]. However, other investigators could not confirm this relationship [45]. The *CYP2E1**6 allele frequencies are 10%, 25%, 33%, 15% and 20% in European–American, Taiwanese, Japanese, Hawaiians and Indian population respectively [46,47]. The frequencies of *CYP2E1*1A/*1A*, **1A/*5* and **5/*5* genotypes have been reported in different populations (Table 1). They are similar in European–Americans, African–American and Indians [47], but vary from those in the Taiwanese population [42]. A report on Taiwanese patients has shown that common **1A/*1A* genotype at *CYP2E1* increased the risk of ATD hepatotoxicity, with OR of 2.52 and a 95% CI of 1.26–5.05, in adult TB patients [42]. The same study also reported that individuals with the **1A/*1A* genotype and NAT2 slow acetylation status had enhanced risk of ATD hepatotoxicity, with an OR of 7.43 and 95% CI of 2.42–22.79, compared with the **1A/*1A* genotype (OR: 2.52; 95% CI: 1.26–5.05) or slow acetylation status alone (OR: 2.3; 95% CI: 1.21–4.39). Although the sample size was low, we also observed that the variant *CYP2E1*6* allele and the **1A-*6-*1D* haplotype at *CYP2E1* increased the risk of hepatotoxicity (OR: 11.0; 95% CI: 1.02–110 and OR: 4.6; 95% CI: 1.3–16.3, respectively) in Indian pediatric patients [48]. This haplotype **1A-*6-*1D* includes the common**1A* allele at (*RsaI* [+].*PstI* [-]) sites, but the variant **6* and **1D* alleles at two other polymorphic sites. Another study on a mixed population of Caucasians, Hispanics, Africans, South Americans and Asians also observed that the common **1A* allele at *CYP2E1* elevated the levels of ATD-induced liver enzymes [39]. However a Korean study did not observe association between ATD hepatotoxicity and *CYP2E1* polymorphism [37].

GSTM1 & *GSTT1* polymorphisms

The GSTs are a family of enzymes known to play important roles in the detoxification of several carcinogens, toxic chemicals and drugs [49,50]. GSTs generally catalyze conjugation reactions between glutathione and the substrates for solubilization and excretion from the body. These enzymes are coded by at least five distinct loci, known as α, μ, π, θ and γ. Of these five loci, *GSTM1* (μ type) and *GSTT1* (θ type) have gained attention in the hepatotoxicity association studies [38,51]. Homozygous deletion at *GSTM1* and *GSTT1* in some individuals of a population results in complete loss of

Table 2. Distribution of *GSTM1* and *GSTT1* homozygous deletion genotypes in different populations.

Locus	Population	*Homozygous deletion (range) (%)*
GSTM1	African–American	23–41
	Chinese	35–63
	Hispanic	40–53
	European–American	35–62
	Pacific Islander & Malaysians	62–100
	India	20–79
GSTT1	African–American	22–29
	Chinese	58
	Hispanic	10–12
	European–American	15–31
	Malaysians	38
	India	3–39
	Korean	42–46

enzymatic activity, but individuals who carry either one or both of the functional alleles possess enzymatic activity [52]. Polymorphisms at these two loci have been extensively studied in different populations to find their association with the risk of different cancers [53]. Frequencies of *GSTM1* and *GSTT1* homozygous deletion genotypes in healthy individuals have been reported in different populations (Table 2) [54–58]. There is a wide range of distribution in different populations. In general, homozygous deletion frequencies are comparatively greater in Pacific Islander, Malaysian, Chinese, Korean and Japanese populations. A few ethnic populations in India also had high frequencies of *GSTM1* and *GSTT1* homozygous deletion genotypes [57].

Glutathione *S*-transferases are proposed to play important roles in the metabolism of ATDs, particularly INH. INH-treated liver injury has been shown to be associated with depletion of hepatic glutathione content and reduction of GST activity [59–61]. Glutathione (GSH) plays a protective role as an intracellular free radical scavenger conjugating with toxic metabolites that are generated from metabolism of INH. Sulfhydryl (SH) conjugation of the metabolites facilitates their elimination from the body and reduces the potential for toxicity. So deficiency of GST activity, because of homozygous deletion at *GSTM1* and *GSTT1* loci, may modulate susceptibility to ATD hepatotoxicity. We have shown that the risk of ATD hepatotoxicity is increased, with an OR of 2.13 and 95% CI of 1.25–3.10, in Indian patients with *GSTM1* homozygous deletion [38]. Similarly, Huang *et al*. also reported that patients with *GSTM1* homozygous deletion had high risk of INH-induced hepatotoxicity, with OR: 2.23; 95% CI: 1.07–4.67, in a Taiwanese population [51]. However, no significant change in risk was observed in patients with *GSTT1* homozygous deletion [38,51]. Moreover, this observation has to be confirmed in different ethnic populations with more samples sizes. Therefore, screening of patients for *GSTM1* homozygous deletion may provide prior information for better control of ATD hepatotoxicity.

Other studied loci

Manganese superoxide dismutase

Drugs are also metabolized by CYP enzymes to produce toxic intermediates and reactive oxygen species (ROS) [40]. Accumulation of ROS from different metabolic reactions could also cause hepatic injury. Mitochondria are a good source of ROS generation during the reactions in the electron transport chain. Manganese superoxide dismutase (MnSOD) is involved in the reduction of ROS load in mitochondria. Huang *et al*. reported that genotypes containing the variant C allele (T>C polymorphism at codon 47 i.e., Ala>Val) increased the risk of ATD hepatotoxicity in Taiwanese patients [51]. It is likely that the MnSOD containing the variant amino acid, Val, at codon 47 increased the generation of toxic hydrogen peroxide, which may cause liver damage.

Human leukocyte antigen alleles

It was reported that immunogenic factors such as human leukocyte antigen (HLA) DR2 molecules were associated with pulmonary tuberculosis in different patient populations [62]. Later Sharma *et al*. reported that the absence of *HLA-DQA1*0102* and presence of *DQB1*0201* alleles were independently associated with increased risk of ATD hepatotoxicity in Indian patients [63].

Conclusions

Several investigators have studied the association between ATD hepatotoxicity and polymorphisms at different drug-metabolizing loci in different populations worldwide. Some studies had reported that NAT2 slow acetylation and the *CYP2E1 *IA* allele increased the risk of ATD hepatotoxicity, although a few studies

could not reproduce this observation. In addition, a limited number of studies have also shown that *GSTM1* homozygous deletion could increase the risk of hepatotoxicity.

Future perspective

It has been reported that only three or four SNPs (at 282 C>T, 341 T>C, 590 G>A and 857 G>A) could predict the acetylation status of 90–99% individuals of a population (Roy P *et al*. unpublished data, [31,64]). These observations are also to be confirmed in different ethnic populations. So, genotyping at three or four SNPs will reduce the cost of knowing the acetylation status of individuals in a population. Since ATD hepatotoxicity is a worldwide problem in the treatment of TB, few developed countries are taking the initiative to genotype the patients at three or four appropriate SNPs on *NAT2* prior to treatment for better control of ATD hepatotoxicity. TB is more prevalent in developing or poor nations, and the disease is highly related to hygiene and nutrition. WHO, along with the state governments, should take initiatives to decrease the load of infected individuals and ATD hepatotoxicity. Following this, it may introduce pharmacogenetic evaluation to control ATD hepatotoxicity on a larger scale to reduce the cost by a high-throughput assay system, probably yet to be developed for hepatic injury. Few studies also reported an association between polymorphisms at *CYP2E1* and the risk of ATD hepatotoxicity [39,42,48]. Like *NAT2*, several polymorphic sites at *CYP2E1* have been reported in different populations [103]. Knowledge should be gained regarding which alleles at these sites will provide the best information regarding the enzymatic activity and whether two or three polymorphic sites, such as *NAT2*, should be sufficient to provide information regarding activity. It is also important to evaluate whether a combination of different risk genotypes/alleles at susceptible loci (such as *NAT2*, *CYP2E1* and *GSTM1*) could impart more risk compared with one or two risk genotypes/alleles. There should be consensus among worldwide investigators to genotype specific polymorphic sites, at *NAT2* and *CYP2E1*, which could modulate enzymatic activities. Then it will also be useful for meta- and pooled analysis in ATD hepatotoxicity association studies.

Published reports have dealt with a relatively small sample size involving one or few loci. Future studies should include a much larger sample of cases and controls (say, 2000 individuals in each group, since more than 1000 samples for a locus with 50% polymorphism is needed to get an odds ratio of 1.25) from different ethnic populations and simultaneous analysis of more loci [65]. Interaction between susceptibility genes and exogenous risk factors (e.g., age, diet and other drugs) should be studied in greater details. Most of the conclusions on genetic susceptibility and ATD hepatotoxicity were drawn from case–control studies. Family-based studies will be more valuable in identifying the predisposing loci, since unaffected individuals in the family may provide ethnically matched controls and nullify most of the confounding factors.

Another important area of TB treatment is gaining research priority in different laboratories. The bacteria that cause the disease also possess nat2 protein for its own function (such as synthesis of cell wall lipid). Since there are dissimilarities in the structure and function between human NAT2 and bacteria nat2 protein, it has been suggested that bacterial nat2 protein may be targeted with drugs, without affecting human NAT2 protein [66], to avoid INH hepatotoxicity.

Executive summary

Antituberculosis drugs & toxicity

- Isoniazid, rifampicin, pyrazinamide and ethambutol are commonly used drugs against TB.
- Isoniazid treatment is associated with elevation of liver enzyme activity and severe hepatotoxicity in some of the patients.
- In slow acetylators, NAT2 acetylates isoniazid slowly with the accumulation of toxic metabolites and manifestation of anti-TB drug (ATD) hepatotoxicity.

Polymorphisms & ATD hepatotoxicity

- NAT2, CYP2E1 and GSTM1 enzymes play important roles in ATD hepatotoxicity.
- NAT2 slow acetylating genotypes are mostly involved in ATD hepatotoxicity.
- The *CYP2E1* common genotype increases the risk of ATD hepatotoxicity.
- *GSTM1* homozygous deletion increases the risk of ATD hepatotoxicity.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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