Chapter 6

Tuberculosis Pharmacogenetics: State of The Art

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1. Introduction

The interindividual variability in the metabolism of xenobiotics and drug response is extensive and many factors are involved with this variation including genetic composition, gender, age, co-administration of medication, individual physiology, pathophysiology and presence of other environmental factors (alcohol consumption, smoking, eating habits).

To produce their therapeutic effects, the drug must be present in appropriate concentrations at its site of action. Although the therapeutic concentrations are dependent on the given dose, they will also depend on the magnitude and rate of absorption, distribution, biotransformation, and excretion. Pharmacokinetics studies the course and distribution of drug and its metabolites in different tissues, covering the mechanisms of absorption, transport, metabolism and excretion. In addition, pharmacodynamics concentrates on the biochemical and physiological effects of drugs and their mechanism of action. Proteins involved in drug effects are defined as target molecules and include not only (direct) receptors, but also proteins associated with mechanism of action such as e.g. signal transducer proteins [1].

After its administration, a drug is absorbed and then distributed throughout the body, requiring the coordinated functioning of various proteins, including metabolic enzymes, trafficking proteins, receptor proteins, and others. Medication can enter the body as either active drugs or as inactive prodrugs. Most drugs are metabolized in the liver to make them more soluble for subsequent elimination through the kidneys or intestines. Prodrugs require metabolic conversion, also called biotransformation, to liberate the active compound. Complete biotransformation of any one drug typically requires several different enzymes. [2]. Genetic variability has been described to have effect on drug absorption and metabolism and its interactions with the receptors. This forms the basis for

slow and rapid drug absorption, poor, efficient or ultrarapid drug metabolism and poor or efficient receptor interactions [3]. The consequences of such variations can lead to adverse drug reaction and/or terapeutic failure.

In this context, pharmacogenetics is the study of genetic variations associated with individual variability in drug response, including differences in efficacy, drug-drug interactions, and the relative risk of an adverse response to drugs. It includes the study of genetic polymorphisms that could affect the expression or activity of drug transporters, drug metabolizing enzymes and drug receptors [2-4].

It's estimated that 99.9% of the human genome sequence between individuals is identical and genetic differences in polulations are called mutations if they are present in less than 1% and polymorphisms when present in at least 1% of a population. A single-nucleotide polymorphism (SNP) involves a replacement of one nucleotide base with any one of the other three and occuring at approximately one out of every 1,000 bases in the human genome [5].

A mutation or polymorphism in genes that encode metabolic enzymes, carriers or receptors can affect the drug pharmacokinetics and pharmacodinamics leading to undesired therapeutic effects. The identification of these genetic markers which predicted if a person responds well or not to a specific drug could help to select the right medication in right dosage, maximizing the eficacy and preventing or reducing the adverse drug reactions.

2. Problem statement

TB is an important global public health problem but has cure in almost 100% of the new cases if correct quimiotherapy is applied. The American Thoracic Society (ATS) treatment guidelines recommend an initial phase for TB treatment which consists of rifampicin 10 mg/kg (maximum 600 mg), isoniazid 5 mg/kg (maximum 300 mg), pyrazinamide 15–30 mg/kg (maximum 2 g), and ethambutol 15–20 mg/kg (maximum 1.6 g) given daily for 8 weeks, followed by a continuous phase of isoniazid 15 mg/kg (maximum 900 mg) and rifampicin 10 mg/kg (maximum 600 mg) administered 2–3 times/week for 18 weeks [6]. The use of fixed-dose combination (FDC) tablets containing anti-TB drugs has been recommended by the World Health Organization (WHO) as an additional measure to improve treatment adherence by reducing the number of tablets to be taken. The principal disadvantages of combining three or more drugs in one tablet include (a) the possibility of overdosage or underdosage resulting from a prescription error, (b) changes in the bioavailability of rifampicin and (c) difficulties in determining which drug is responsible for adverse effects [7].

Isoniazid (INH) is an important drug in the TB treatment and was introduced in chemotherapic scheme since 1952. It is the hidrazine of isonicotinic acid and shows cytotoxic activity for *Mycobacterium tuberculosis* both in rest (during latency) and proliferation phases. This drug enters easily in macrophague cells to kill bacilli in multiplication and is specific for mycobacteria [1].

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INH-induced adverse reactions include fever, nausea, vomiting, hepatotoxicity, skin reactions, gastrointestinal and neurological disorders. Only in the early 1970s, the occurrence of severe liver injury as a side effect of this drug was recognized, resulting in the death of some patients [8]. Among the first-line anti-TB drugs, INH is the main associated with drug-induced hepatotocixity with a frequency ranging from 1 to 30% in different populations [9]. Other drugs causing liver injury are mainly reported in combination with INH [10, 11]. Drug-induced hepatotoxicity is defined as a serum alanine aminotransferase (ALT) level three times greater than the upper limit of normal (ULN) with clinical symptoms or five times the ULN without symptoms. In both cases treatment should be interrupted and, generally, a modified or alternative regimen is introduced [9]. Because these adverse reactions do not only affect morbidity and mortality rate but also lead to treatment interruptions, failure and relapse, adverse reactions contribute to the spread of the disease and the emergence of multidrug resistence (MDR).

Adverse Drug Reactions (ADRs) are common causes of hospitalization and lead to large costs to society. There are two main financial burdens due to illnesses caused by ADRs: that of treating and that of avoiding them [12]. The occurrence of serious and fatal ADRs has been extensively studied in hospitalized patients and a meta-analysis of prospective studies in approximately forty hospitals in the United States of America (USA) suggests that 6-7% of hospitalized patients suffer from serious ADRs and 0.32% of patients develop fatal ADRs [13]. This results in approximately 100,000 deaths annually in the U.S. and an annual cost of over a hundred billion dollars to the society due to prolonged hospitalization and reduced productivity [3, 13]. Furthermore, it has been estimated that ADRs are responsible for up to 7% of all admissions in hospitals in the United Kindown (UK) and 13% in medical clinics in Sweden [3], which shows the magnitude of this problem in the context of chemotherapy and drug development. Additionally, in France, a 10-year study in the Liver Unit of Hôpital Beaujon in Paris showed that among all patients hospitalized with acute hepatitis, 10% were due to adverse reaction to drugs and the prevalence of drug hepatotoxicity in patients older than fifty years exceeded 40%. In Japan and other Eastern countries, drugs are responsible for about 10-20% of cases of fulminant hepatitis [14].

Liver injury is the most common ADR and the main complication during chemotherapy since liver is the central organ for the biotransformation and excretion of most drugs and xenobiotics [14-17]. There are basically six mechanisms involving primarily the hepatocyte injury. The reactions of mono-oxygenase cytochrome P450 (CYP450) with certain drugs generate toxic metabolites that bind to intracellular proteins, leading to calcium homeostasis pump dysfunction with consequent disruption of actin fibers and cell lysis. Some drugs affect transport proteins in the cell membrane interrupting the flow of bile and then causing cholestasis. Several reactions involving CYP P450 can promote binding of the drug to the enzyme, with consequent exposure of this complex on the cell surface for recognition by T cells and antibody production as part of the autoimmune response. Finally, certain drugs may promote hepatic injury mediated by programmed cell death (apoptosis) or being capable of inhibiting respiration and/or mitochondrial beta-oxidation [17].

Xenobiotics are usually lipophilic and this facilitates their transport in association with lipoproteins in the blood stream and their penetration of lipid membranes and entrance into organs. However, physicochemical properties of drug molecules difficult their removal from the organism by biliary or renal excretion and therefore, these substances require enzymatic conversion to water soluble compounds [1]. The xenobiotics metabolization, often through multiple pathways, can generate metabolites that are more toxic than the substrate and through their interaction with target macromolecules such as DNA, RNA, proteins and receptors, generate the toxic effects. The organ affected is generally that reponsible for drug metabolization or excretion of metabolites [1].

The enzyme systems responsible for the biotransformation of many drugs are located in the endoplasmic reticulum of the liver (microsomal fraction). Such enzymes are also present in the kidneys, lungs and gastrointestinal epithelium, although at a lower concentration [1]. The metabolic modification in biotransformation usually takes place in two consecutive steps and results in the loss of biological activity. Phase I reactions convert the xenobiotic into a metabolite with higher polarity by oxidation, reduction or hydrolysis and generates a pharmacologically inactive or less active, or in the case of a pro-drug, more active molecule. This metabolite is than either eliminated or go through Phase II reactions (so-called synthesis or conjugation reactions), involving binding to a primary metabolite or endogenous substrate such as glucuronate, sulfate, acetate, amino acids or glutathione (tripeptide). Such enzymatic reactions include glucuronidation, methylation, sulfation, acetylation, conjugation with glutathione and conjugation with glycine [1].

The risk for developing hepatotoxicity is associated both with genetic and acquired factors. The acquired factors include: age, gender, nutritional habits, drug abuse, pregnancy and extrahepatic disease. Genetic variations in isoenzymes involved in drug biotransformation can result in abnormal reactions leading to toxic effects [14,17]. In the case of INH in particular, advanced age is a risk factor for hepatotoxicity whereas deficiency in the ability of N-acetylation represent a genetic risk factor for liver injury.

INH is administered orally and rapidly absorbed through the gastrointestinal tract passing through the liver by the portal venous system before reaching the general circulation where is metabolized by a process known as the first pass effect with reduction of its biodiponibility. About 75% to 95% of the INH is excreted by the kidneys during the first 24 hours, mainly as the metabolic forms acetyl-isoniazid and isonicotinic acid [1].

In the liver, INH is metabolized to acetylisoniazid by N-acetyltransferase 2 (NAT2), followed by hydrolysis to acetylhydrazine and then oxidized by cytochrome P4502E1 (CYP2E1) to hepatotoxic intermediates [18, 19]. These metabolites can destroy hepatocytes either by interfering with cell homeostasis or by triggering immunologic reactions in which reactive metabolites that are bound to hepatocyte plasma proteins may act as haptens [17]. The other metabolic pathway to generate toxic metabolites is direct hydrolysis of INH to hydrazine, a potent hepatotoxin. NAT2 is also responsible for converting acetylhydrazine to diacetylhydrazine, a nontoxic component [18, 20, 21] (Figure 1). Glutathione S-transferase (GST), an important phase II detoxification enzyme, is thought to play a protective role as an intracellular free radical scavenger, which conjugates glutathione with toxic metabolites that are generated

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from CYP2E1 [22]. Sulphydryl conjugation facilitates the elimination of metabolites from the body and reduces the toxic effect [23] (Figure 1).

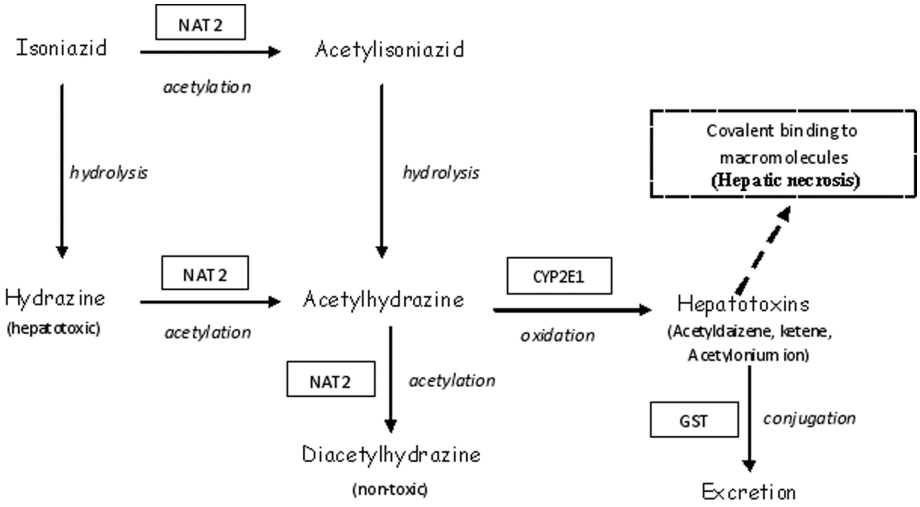

Figure 1. Schematic representation of the INH metabolism. The major enzymes involved in this pathway are indicated in boxes [20, 24].

In the last few years, an increasing number of studies have suggested that genetic polymorphisms in *NAT2*, *CYP2E1* and *GST* genes would be associated with susceptibility to drug-induced hepatotoxicity during TB treatment. The present work focused in an overview of the role of such polymorphisms in occurence of liver injury induced by anti-TB drugs, and by INH in particular.

3. State of the art

3.1. N-acetyltransferase 2

NAT2, the main enzyme responsible for the metabolism and inactivation of INH in humans, is a Phase II enzyme that catalyzes the transfer of the acetyl group from the cofactor acetyl coenzyme A (acetyl-CoA) to the nitrogen terminal of the drug. Variations in activity of NAT2 were discovered over 50 years ago when observing interindividual differences in the metabolism of INH and the level of drug-induced toxicity in TB patients. NAT2 is encoded by the *NAT2* gene and according family genetic studies, variability of *NAT2* was directly related to the emergence of different phenotypes of acetylation [25].

The molecular study of human N-acetyltransferases revealed the presence of three genetic loci, two very homologous encoding the enzymes NAT1 and NAT2, and a third including the

pseudogene *pNAT* (Figure 2). These loci are located on chromosome 8 between 170-360Kb at 8p22 [26]. The *pNAT* is a pseudogene containing a premature stop codon, and is not transcribed. *NAT1* and *NAT2* genes consist of 873 bp, are intronless, and encode proteins of 34 kDa. Protein sequence homology between both enzymes is 81% while that between their respective genes is 87%. Both enzymes have N-acetylation, O-acetylation and NO-transfer in different xenobiotics and carcinogens but differ considerably in their tissue distribution and expression levels during embryonic development [26-28].

Both *NAT1* and *NAT2* are polymorphic genes and SNPs in their coding region can alter the enzymatic activity [29, 30] and are the basis of the three major genetically determined phenotypes, being rapid, intermediate and slow acetylators, which are inherited as a codominant trait [31, 32]. The reference *NAT2*4* allele (without mutations / wild-type) and 66 variants were identified and classified in human populations depending on the combination of up to four SNPs present throughout the *NAT2* coding region [33]. So far, over 30 SNPs have been identified in this region, including several rare mutations described in different populations [34]. Among these, the seven most frequent are the 191 G>A (R64Q), 282 C>T (silent), 341 T>C (I114T), 481 C>T (silent), 590 G>A (R197Q), 803 A>G (K268R) and 857 G>A (G286T) SNPs identified in different human populations [35]. *NAT2* alleles containing the 191G>A, 341T>C, 590G>A or 857G>A SNPs are associated with slow acetylator *NAT2* alleles [33].

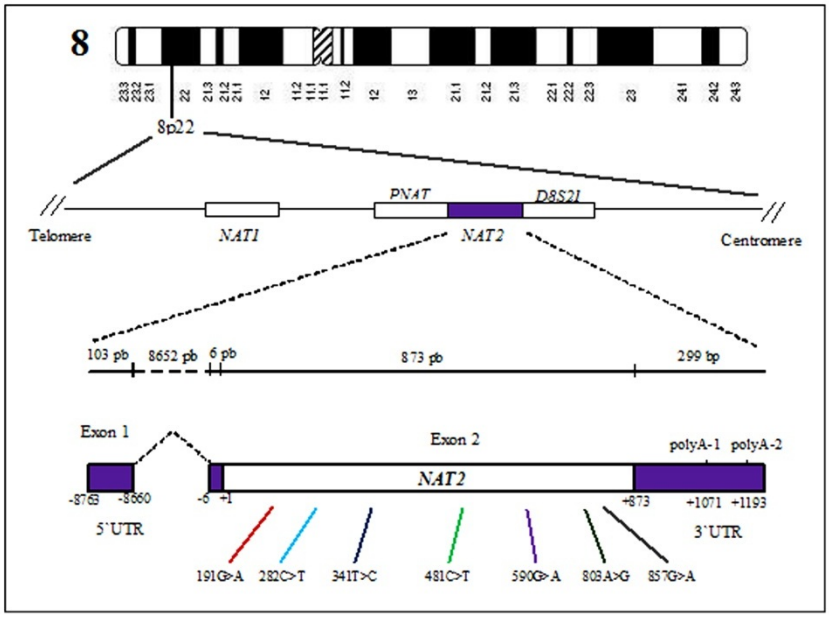

Figure 2. Schematic representation of *NAT* genes on human chromosome 8p22. Distribution of the seven most common SNPs in *NAT2*. D8S21 represents a polymorphic marker situated in the *NAT2* locus [26, 36].

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Presence of different SNPs in *NAT2* can be easily determined by genotyping procedures such as PCR-RFLP [37], allele specific PCR [38] or direct sequencing [39]. To achieve the *NAT2* genotype of each individual and predict the phenotype, the haplotype of both chromosomes is usually reconstructed using the statistic software (PHASEv2.1.1[40, 41]). Using haplotype data, many studies have reported the frequencies of the different acetylation profiles among ethnically different populations showing the high diversity around the world. In Asians and Ameridians, the fast acetylator phenotype is more frequent [42-44] whereas in Euro-descendants slow acetylators account for 50% of the study population [37,45]. The molecular basis for such discrepancy is that the most common *NAT2* allele in Euro-descendants is very rare in Asians and may represent a different selective advantage within the gene pools of these separate populations. Description of new alleles of *NAT2* is still ocurring in recent studies [34].

In an attempt to establish an association between acetylation profiles and development of disease, cohort or case-control studies have been performed using of genotyping and phenotyping tools. Evidence was found for an association between the slow acetylator predicted phenotype and developing urinary bladder cancer, while rapid acetylators seem more susceptible to development of colon cancer. For a review, see [27, 46].

For many years, INH has been considered the main cause of hepatotoxicity during TB treatment and association studies between the acetylation phenotypes and susceptibility to liver-related ADRs have been performed. Two early studies conducted in oriental populations investigated the association of the acetylator phenotype with INH induced hepatotoxicity and observed an increased risk of developing hepatotoxicity by INH among the slow acetilators [47, 48]. This observation was confirmed in several other studies performed in different populations [49-52].

Several studies reported the absence of a relationship between acetylation status and hepatotoxicity during TB treatment [53-55] but some, suggested the rapid acetylators as more susceptible to side effects [55, 56]. Reasons for these different findings range from genotyping methods to ethnicity. In some studies, NAT2 acetylation phenotypes were determined by an enzymatic method leading to possible misclassification of the acetylation status [53, 56, 57]. Indeed, it is difficult to compare the accuracy of different NAT phenotyping methods or different cut-off points using the same phenotyping method. In addition, for genotyping, investigators sometimes select a small number of SNPs to define the acetylation status [54, 55]. Since the frequencies of *NAT2* alleles are different among worldwide populations and new alleles are been identified in some countries, investigators need to characterize such alleles in their own study population in order to choose appropriate SNPs for genotyping and classify the acetylation status of individuals, otherwise overestimation of slow acetylators may be obtained, contributing to a spurious results in the association study.

Recently, a study with an admixed population showed that *NAT2* is a genetic factor for predisposition to anti-TB drug-induced hepatitis. In this case, *NAT2* genes were well characterized by direct sequencing and their genotypes achieved by haplotype reconstruction using the PHASE software. In addition, functional unknown genotypes were disconsidered and others confounding variables for hepatotocixity were taken into account. The incidence of elevated levels of serum transaminases was significantly higher in slow acetylators than those

of the rapid/intermediate type. These results corroborate with the current hypothesis that the acetylator status may be a risk factor for the hepatic side effects of isoniazid [58].

Finally, a meta-analysis was conducted to solve the problem of inadequate statistical power and controversial results based on accumulated data with small sample size [59]. Data from 14 studies performed between 2000 and 2011 were pooled and showed that TB patients with a slow acetylator genotype had a higher risk of anti-tuberculosis drug induced hepatotocixity than patients with rapid or intermediate acetylation ($p < 0.001$). Moreover, subgroup analyses indicate that both Asians and non-Asians slow acetylators develop anti-tuberculosis drug induced hepatotocixity more frequently. Additionally, there were statistically significant associations between NAT2*5/*7, NAT2*6/*6, NAT2*6/*7 and NAT2*7/*7 and the risk of anti-TB drug induced hepatotocixity [59].

As a final consideration, NAT acetylates more slowly not only isoniazid but also acetylhydrazine, the immediate precursor of toxic intermediates, to the harmless diacetylhydrazine [60, 61]. This protective acetylation is further suppressed by INH competition. Therefore, slow acetylators may be prone to higher accumulation rates of INH toxic metabolites. Another important route to generate toxic intermediates is the direct hydrolysis of unacetylated INH [62], producing hydrazine that also induces hepatic injury [62, 63]. Pharmacokinetic studies showed that the serum concentration of hydrazine was significantly higher in slow acetylators than in rapid acetylators, probably due to the high INH concentration. The high amount of INH disposed of through this pathway is likely to lead to enhanced hydrolysis to hydrazine, since the rate of metabolic conversion of INH to acetylisoniazid is lower in slow than in rapid acetylators [64, 65]. All of these drug-disposal processes may support the finding that slow acetylators are prone to INH-induced hepatitis. We therefore conclude that screening of patients for the *NAT2* genetic polymorphisms can prove clinically useful for the prediction and prevention of anti-tuberculosis drug induced hepatotoxicity.

3.2. CYP450

Cytochromes P450 (CYP450) are hemoproteins and form the most important enzymatic group for Phase I biotransformation. The main activity of isozymes of CYP450 system is oxidation and they are located in the smooth endoplasmic reticulum, mainly in liver cells. However, these mono-oxygenases are also localized in the intestine, pancreas, brain, lung, kidney, bone marrow, skin, ovary and testicles [66]. The CYP450 proteins are clustered into families and subfamilies according to the similarity between the amino acid sequences: where family members have ≥ 40% identity in amino acid sequence, members of the same subfamily share ≥ 55% identity [67].

The CYP450s are responsible for the metabolization of several endogenous substrates and the synthesis of hydrophobic lipids such as cholesterol, steroid hormones, bile acids and fatty acids. Moreover, some enzymes of P450 complex metabolize exogenous substances including drugs, environmental chemicals and pollutants as well as products derived from plants. The metabolism of exogenous substances by CYP450 usually results in detoxification of the xenobiotic; however, the reactions triggered by such enzymes can

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lead to generation of toxic metabolites that contribute to the increased risk of developing cancers and other toxic effects [68].

The complete sequencing of the human genome revealed the presence of about 115 genes of CYP450, including 57 active genes and 58 pseudogenes [67]. They belong to families 1-3 and are responsible for 70-80% of Phase I-dependent metabolism of clinically used drugs. Other families of CYPs are involved in metabolism of endogenous components [66]. The CYP2 constitutes the largest family of isoenzymes and comprises one third of all human CYPs. Genes encoding these enzymes are polymorphic and the frequency distribution of allelic variants in different ethnic groups differs. Overall, four phenotypes based on genotypes can be identified: (i) poor metabolizers who present low enzymatic activity, (ii) intermediate metabolizers, usually heterozygous for a defective allele, (iii) rapid metabolizers, who have two normal alleles and (iv) ultrarapid metabolizers, who have several gene copies [69].

The enzyme CYP2E1 is expressed mainly in the liver but can be found in other organs such as kidney, gastrointestinal tract and brain and involved in oxidation of substrates such as ethanol and the metabolism of many drugs and pre-carcinogens. Besides ethanol, CYP2E1 can be induced by various drugs such as INH but also by hydrocarbons, benzene, chloroform and various organic solvents [70].

The activity of CYP2E1 is also modulated by polymorphisms in several locations of its gene and more activity of this enzyme may increase the synthesis of hepatotoxins. Two polymorphisms upstream of the *CYP2E1* transcriptional start site are characterized by *Pst* I and *Rsa* I digestion and appear to be in complete linkage disequilibrium (Figure 3). These two polymorphisms are located in a putative HNF-q binding site and thus may play a role in the regulation of *CYP2E1* transcription and subsequent protein expression [71]. Genotypes of *CYP2E1* are classified as being *1A/*1A, *1A/*5 or *5/*5 by *Rsa* I based restriction analysis. The polymorphism detectable by *Dra* I (7632 T>A) is located in intron 6 and characterizes the allelic variant *CYP2E1*6*. The other polymorphism is an insertion/deletion of 96 bp (*CYP2E1*1D* and *1C alleles) that regulates the expression of the gene [72]. Some studies have shown that allelic variants *CYP2E1 *5*, *6 and *1D would increase enzyme activity [71, 73]. However, other authors did not confirm any relationship with these polymorphisms with CYP2E1 activity [74].

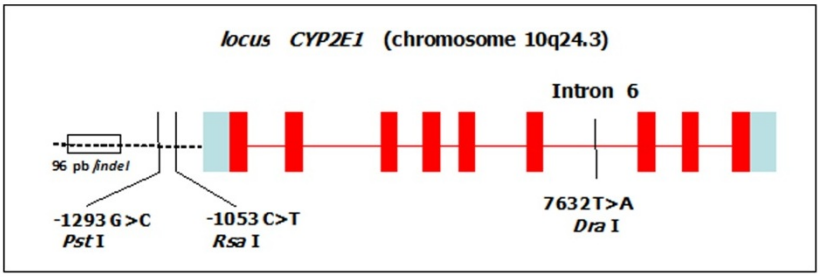

Figure 3. Polymorphic and corresponding restriction enzyme cutting sites at *CYP2E1* [24].

Several studies have described the involvement of polymorphisms in *CYP2E1* in cancer development but results are controversial. The studies showed that the frequency of SNP -1053 C>T in the promoter region varies significantly in different ethnic groups. The mutant allele is present with a frequency of 2-8% in Euro-descendants but varies in Asia from 25 to 36% [75].

In 2003, Huang and coworkers showed an association of the wild-type genotype *1A/*1A with risk of developing liver damage induced by isoniazid in adult TB patients, regardless of their profile of acetylation (OR 2.52; 95% CI 1.26 to 5.05) [76]. Later, Vuilleumier and colleagues showed association between this CYP and isoniazid-induced hepatotoxicity, without hepatitis, during chemoprophylaxis for TB (OR 3.4; 95% CI 1.1 to 12; p = 0.02). The risk of having high levels of liver enzymes was 3.4-fold higher when compared with all other *CYP2E1* genotypes [55]. Another study on Indian children with TB showed association between risk of hepatotoxicity and polymorphisms in *CYP2E1*, despite of low sample size [77]. However, a study with on a Korean population found no relationship between hepatic adverse effects with genotype *1A/*1A of *CYP2E1* during anti-TB treatment [51]. Lack of association between this CYP and antituberculosis drug-induced liver injury was also observed in Brazil [58]. The discrepancy of these results may be due to differences in the frequencies of *CYP2E1*1A* and *CYP2E1*5* alleles among the populations and the different criteria to define hepatotoxicity used.

Finally, CYP2E1 converts acetyl hydrazine into hepatotoxins like acetyldiazene, ketene and acetylonium ion. The reaction of acetyl hydrazine (at high levels) with CYP2E1 leads to covalent binding of these secondary metabolites with intracellular proteins (Figure 1). As a consequence, intracellular changes occur resulting in loss of ionic gradients and decrease of ATP levels and consequent disruption of actin followed by cell lysis. Further studies in different populations and with a larger sample size are needed to determine the true influence of CYP2E1 gene polymorphisms on the occurrence of liver injury during treatment for TB.

3.3. Glutathione S-transferases

Glutathione S-transferases constitute a superfamily of multifunctional ubiquitous enzymes that play an important role in cellular detoxification by protecting macromolecules against reactive electrophilic attack. The GSTs are Phase II enzymes that catalyze the nucleophilic attack of glutathione (GSH) into components that contain an electrophilic carbon, nitrogen or sulfur atom. The combination of the GSH with these compounds often leads to formation of less reactive and more water soluble products, more easily excreted by the body [23, 78].

Glutathione transferases are of great interest to pharmacologists and toxicologists, since they are drug targets for the treatment of asthma and cancer, in addition to metabolize drugs, insecticides, herbicides, carcinogens and products of oxidative stress. Polymorphisms in *GST* genes are often correlated with susceptibility to various cancers, as well as alcoholic liver disease [23, 78-81].

In humans, eight gene families of soluble (or cytosolic) GSTs have been described: alpha (α) located on chromosome 6, mu (μ) on chromosome 1, theta (θ) on chromosome 22, pi (π) on chromosome 11; zeta (ζ) on chromosome 14, sigma (σ) on chromosome 4; kappa (κ) (chromo-

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somal location not given) and omega (Ω) on chromosome 10 [80]. This classification is based on amino acid sequences, substrate specificity, chemical affinity, protein structure and enzyme kinetics. These enzymes are highly expressed in the liver and constitute up to 4% of total soluble proteins but can be seen in several other tissues [82]. GSTs have an overlap of specific substrates and the deficiency in one isoform can be compensated by other isoforms. Glutathione S-transferase mu (GSTM), glutathione S-transferase theta (GSTT) and glutathione S-transferase Pi (GSTP) have been the most studied isoform [83-88].

The subfamily GST mu is encoded by five genes arranged in tandem (5_-*GSTM4-GSTM2-GSTM1-GSTM5-GSTM3*-_3), forming a 100 kb gene cluster on chromosome 1p13.3 (Figure 4). Polymorphisms have been identified and clinical consequences of genotypes resulting from combinations of alleles *GSTM1*0*, *GSTM1*A*, and *GSTM1*B* have been widely investigated [78, 81, 89, 90]. Individuals who possess the homozygous null for *GSTM1* (GSTM1*0/GSTM1*0) do not express this protein. Thus, the absence of this gene can cause an increased accumulation of reactive metabolites in the body, increasing the interaction with cellular macromolecules and tumor initiation process. *GSTM1*A* and *GSTM1*B* differ in only one base in exon 7 and encode monomers that form active dimers. The catalytic activity of these enzymes are very similar [91].

The *GSTM1* gene is flanked by two almost identical 4.2-kb regions. *GSTM1*0* originates from homologous recombination between the two repeat regions which results in a 16 Kb deletion containing the entire gene *GSTM1* (Figure 4). *GSTM1* is precisely excised leaving the adjacent *GSTM2* and *GSTM5* genes intact [78]. In a study of liver specimens of 168 autopsied Japanese subjects, observed was that the *GSTM1*0* null allele was more frequent in livers with hepatitis and hepatocellular carcinoma compared to control livers [92].

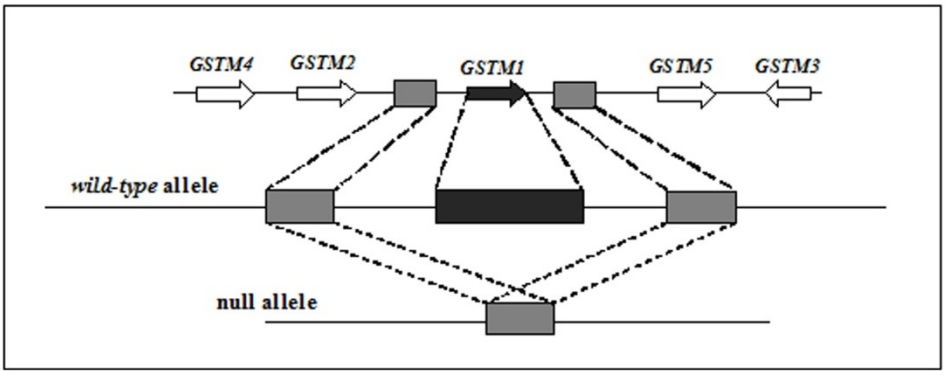

Figure 4. Structural localization of 100 kb gene cluster encoding the GST mu subfamily (chromosome 1p13.3). The figure indicates the homologous recombination event that can happen causing the null allele (*GSTM1*0* - no *GSTM1*). Figure adapted from [78].

The subfamily GST theta consists of two genes, *GSTT1* and *GSTT2*, located on chromosome 22q11.2 and separated by approximately 50 Kb (Figure 5). Analysis of the 119 Kb portion

containing these genes revealed two regions flanking *GSTT1*, HA3 and HA5, with more than 90% homology. HA3 and HA5 contain two identical 403-bp repeats and the occurence of *GSTT1*0* allele is probably caused by homologous recombination between the two regions [78]. In humans, *GSTT1* is also expressed in erythrocytes and probably plays a global role in early detoxification of xenobiotics and carcinogens.

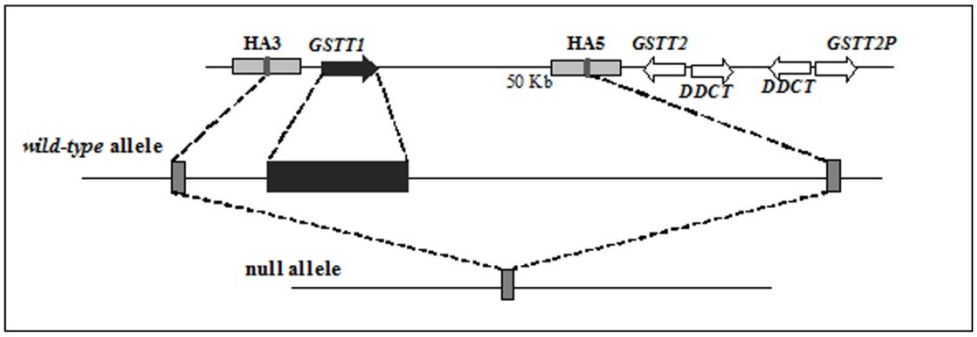

Figure 5. Structural localization of gene cluster encoding the GST subfamily theta (chromosome 22q11.2). The *GSTT1* null allele (*GSTT1*0*) arises by homologous recombination of the left and right 403-bp repeats, which results in a 54-kb deletion containing the entire *GSTT1* gene. Figure adapted from [78].

Deficiencies in the GST activity due to the null genotypes of *GSTM1* and *GSTT1* may modulate susceptibility to the development of hepatotoxicity induced by drugs and xenobiotics. Furthermore, it was observed that the frequencies of *GSTT1*0* and *GSTM1*0* alleles vary within different ethnic groups [78, 82]. Liver injury induced by INH has been associated with the depletion of glutathione content and reduction of GST activity in an animal model for hepatotoxicity by anti-TB drugs [22].

In 2001, Roy and colleagues demonstrated that individuals, homozygous for the null *GSTM1,* had a relative risk of 2.12 for developing hepatotoxicity induced by anti-TB drugs. However, these authors found no association of the *GSTT1* null genotype with this side effects [54]. Similarly, another study in the Thai population found that only the *GSTM1* null genotype increases the risk of liver injury (OR 2.23, 95% CI 1.07 to 4.67) [93]. The opposite was observed by Leiro and colleagues: individuals with the *GSTT1* null genotype had an increased risk of developing hepatotoxicity induced by anti-TB drugs and no significant association was observed between GSTM1*0/*0 genotype and liver injury [94]. These studies suggest a protective effect of glutathione S-transferases to the hepatotoxic effects of isoniazid.

On the other hand, recent studies in different population showed no relationship between GSTM1*0/*0 or GSTT1*0/*0 genotypes and liver injury during anti-TB treatment [58, 95, 96]. In a population-based prospective antituberculosis treatment coort in China, a more robust case-control study was conducted and there was no statistically significant association between null genotypes and hepatotoxicity induced by anti-TB drugs [97].

These controversal results may be due to the small sample size in many studies and the different frequencies of the null genotypes. New populations should be evaluated with large sample size to see which of these polymorphisms can be used as genetic markers for the risk of side effects during anti-TB treatment.

4. Conclusion

The concept of personalized medicine is not really new, but it has been receiving increasing attention in recent years for improval of drug regulation and medical guidelines. There is considerable interindividual variability in metabolism, partly due to human differences on a genetic level. Genetic polymorphisms in drug-metabolizing enzymes can affect enzyme activity and may cause differences in treatment response or drug toxicity, for example, due to an increased formation of reactive metabolites. Such polymorphisms may explain differences in incidence of anti-TB drugs induced hepatotoxicity between different populations.

Genotyping cannot completely predict the phenotype on an individual level because of to the additional contribution of epigenetic, endogenous and environmental factors. However, pharmacogenetics is able to add important information in many cases where therapeutic drug scheme is inappropriate or not sufficient. Nowadays, we can cite three examples of personalized medicine application in clinical practice, (i) AIDS treatment (abavir / skin hypersensitivity / *HLA-B*5701*), (ii) anticoagulation (warfarin / bleeding / *CYP2C9*) and (iii) treatment of acute lymphoblastic leukemia (azathioprine / treatment resistence / *TPMT*) [98].

Although limited information exists regarding isoniazid concentrations that cause toxic reactions, it has been proposed to adjust isoniazid dosage depending on individuals acetylator status: a lower dosage for slow acetylators to reduce the risk of liver injury and a higher isoniazid dosage for fast acetylators to increase the early bactericidal activity and thereby lower the probability of treatment failure [50]. However, more robust clinical prospective studies are needed to evaluate the real contribution of these different polyporphirms in the occurence of liver side effects during anti-TB treatment. Future studies should include larger sample size, different ethnic population, simultaneous analysis of different genetic markers, different degrees of liver injury and consideration of possible confounding factors.

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