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# Polymorphisms in *CYP2E1, GSTM1* and *GSTT1* and anti-tuberculosis drug-induced hepatotoxicity

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**ABSTRACT**

Anti-tuberculosis drug-induced hepatitis (ATD- induced hepatitis) has been linked to polymorphisms in genes encoding drug metabolizing enzymes. *N*-acetyltransferase 2 (NAT2), cytochrome P450 2E1 (CYP2E1*)* and glutathione *S*-transferase (loci *GSTM1* and *GSTT1*) are involved in the metabolism of isoniazid, the most toxic drug for the treatment of tuberculosis (TB). This study was designed to determine the frequency and to evaluate whether polymorphisms at *CYP2E1, GSTM1* and *GSTT1* genes are associated with drug response, as well as to identify clinical risk factors for ATD-induced hepatitis. A total of 245 Brazilian patients undergoing treatment for TB were genotyped using polymerase chain reaction and restriction fragment length polymorphism and sequencing methods. The frequencies of the *CYP2E1* polymorphic alleles *Rsa*I, *Pst*I and *Dra*I are 8%, 8.5% and 12%, respectively. *GSTM1* and *GSTT1* genes are deleted in 42.9% and 12.4% of the population, respectively. Fifteen patients (6.1%) developed hepatotoxicity. Clinical (HIV, female sex and extrapulmonary TB) and genetic characteristics (*CYP2E1* without any mutations, having *NAT2* slow acetylator profile) are at higher risk of developing ATD-induced hepatitis in this population. Genotyping for *GSTM1* and *GSTT1* showed no influence on drug response.

**Key words:** hepatotoxicity, isoniazid, polymorphisms, tuberculosis.

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## INTRODUCTION

Anti-tuberculosis drug-induced hepatitis (ATD-induced hepatitis) is an important clinical problem, being the most severe adverse effect during tuberculosis (TB) treatment (Tostmann et al. 2008, Walker et al. 2009, Cai et al. 2012). Currently, the 6-month therapy consists in the combination of the drugs isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and ethambutol (EMB) (Stine et al. 2013). The last one was added to the Brazilian first-line therapy in 2009 (Conde et al. 2009). Patients often suffer adverse drug reactions (ADR) and it is of increasing concern the fact that hepatotoxicity may be due to additive or synergistic effects of INH and RMP (Yew and Leung, 2006). The frontline drug, INH, has been extensively studied (Cheng et al. 2013). In the human liver, INH is first acetylated by *N*-acetyltransferase 2 (NAT2) into acetylhydrazine, then oxidized into toxic intermediaries by cytochrome P450 2E1 (CYP2E1) (Huang et al. 2003, Chamorro et al. 2013). The toxic compounds produced are detoxified by further acetylation by NAT2 and by conjugation reactions catalyzed by glutathione *S*-transferase enzymes (GST). Frequency and severity of anti-TB drug-induced hepatotoxicity is variable and unpredictable. Secondary pathways and the metabolizing profile take part in the unbalance between hepatotoxin production and liver detoxification (Roy et al. 2001, Leiro et al. 2008). Our previous study of *NAT2* polymorphism demonstrated that a slow acetylation profile is associated to ATD-induced hepatitis (Possuelo et al. 2008).

Risk factors for ATD-induced hepatitis, such as HIV, hepatitis B and C coinfection, elevated baseline transaminases, older age and female sex (Fernández-Villar et al. 2004, Saukkonen et al. 2006, Possuelo et al. 2008, Yimer et al. 2011), are usually implicated, but seem to vary among populations.

Genetic factors can also have a large influence on drug response. Enzymes from cytochrome P450 (CYP) are responsible for about 80% of all phase I drug metabolism (Eichelbaum et al. 2006). *CYP* genes are highly polymorphic and the polymorphisms have been associated with altered gene expression and drug adverse reactions (Ingelman Sundberg, 2004, Ingelman Sundberg et al. 2007). Polymorphisms in *CYP2E1* have been investigated in relation to alcoholism, chronic obstructive pulmonary disease, alcoholic liver cirrhosis, innumerous types of cancer and ATD-induced hepatitis (Kato et al. 1992, Arif et al. 2007, Boccia et al. 2007, Cho et al. 2007, Liu et al. 2007, Khan et al. 2009).

In addition to *CYP2E1, GSTM1* and *GSTT1* enzymes are also involved in the detoxification pathway of INH toxic metabolites. Deficiency in GST activity caused by gene deletion may modulate susceptibility to drug and xenobiotic-induced hepatotoxicity (Roy et al. 2001).

Genetic-environmental factors differ among individuals and populations. The characterization and study of the geographical distribution of human genetic diversity is crucial for the understanding of human genomic variations. The interethnic admixture observed in the Americas makes these populations especially valuable for a pharmacogenomics approach (Suarez-Kurtz and Pena 2006).

This study was designed to determine the frequency and to evaluate whether polymorphisms at *CYP2E1, GSTM1* and *GSTT1* genes are associated with drug response, as well as to identify clinical risk factors for hepatotoxicity, in a population undergoing treatment for TB with isoniazid, rifampicin and pyrazinamide (RHZ) from the city of Porto Alegre, state of Rio Grande do Sul, Brazil.

## MATERIALS AND METHODS

### Study Population

This prospective study was carried out between August 2005 and August 2007 and 245 unrelated patients with newly diagnosed TB from the outpatient section of Hospital Sanatório Partenon (HSP), a public TB reference hospital located in Porto Alegre

(Rio Grande do Sul), were consecutively selected for the study. Inclusion criteria were: patients aged 18 years and older that were newly diagnosed with active TB and were submitted to a daily treatment with INH, RMP, and PZA for the first 2 months, followed by INH and RMP for an additional 4 months. Exclusion criteria were: patients using anti-TB drugs prior to study enrollment, patients whose results of liver function tests prior to the beginning of treatment were higher than twice the upper limit considered normal (ULN) and refusal to participate in the study. Details about treatment, liver function tests and serology for Hepatitis C virus (HCV), Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) are described in Possuelo et al, 2008. Criteria for the diagnosis of ATD-induced hepatitis was an elevation in liver function tests, aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) of more than threefold the ULN (reference values: 40 and 65 U/L, respectively) and/or total bilirubin up to >2.0 mg/dL in the presence of gastrointestinal symptoms such as anorexia, nausea, vomiting and/or jaundice, with serum ALT level normalization after anti-TB drug discontinuation. These criteria are routinely used by pneumologists and gastroenterologists of HSP and are consistent with the recommendations of the Brazilian Tuberculosis Consensus (Castelo Filho et al. 2004). Hepatotoxicity analysis based on the criteria of the International Consensus meeting (Bénichou 1990) (ALT higher than twofold the ULN) for drug-induced hepatotoxicity was also carried out. Clinical and epidemiological data, such as age, sex, skin color (self-reported), alcohol abuse (according to CAGE criteria Mayfield et al. 1974), and use of highly-active antiretroviral therapy (HAART) or another co-medication, were collected using a standardized questionnaire at an interview and the review of medical records of each patient.

The protocol used in the present study was approved by the Research Ethics Committee of the School of Public Health, state of Rio Grande do Sul (protocol number 156/05) and by the Fundação Estadual de Produção e Pesquisa em Saúde- FEPPS (protocol number 18/2006). All patients recruited in the present study signed a written informed consent form. We declare that the experiments comply with current Brazilian laws.

### *CYP2E1, GSTM1,GSTT1* and *NAT2* GENOTYPING

Genomic DNA was extracted from a whole blood sample of each patient according to the *Salting Out* method, modified from Miller et al. (Miller et al. 1988). Polymorphisms in *CYP2E1* were detected using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Molecular Probes, Invitrogen Detection Technologies, OR, USA). Amplification was performed in a PTC-100$^{TM}$ thermocycler (MJ Research Inc) and PCR details are described in Supplementary Table I. After amplification, the fragment was digested with *Rsa*I (5U New England BioLabs) and *Pst*I (4U, Invitrogen, Brazil) endonucleases, at positions -1053C>T and -1293G>C, respectively. A region in intron 6 of *CYP2E1 (*7632T>A) was amplified and digested with *DraI* (8U, New England BioLabs). Wild type *CYP2E1* allele (*1A) has restriction sites for *Rsa*I and *Dra*I, but not for *Pst*I. The presence of restriction sites yielded two fragments of 352 and 143 bp for *Rsa*I restriction digest, 377 and 118 for *Pst*I, and 268 and 306 for *Dra*I restriction digest. Digested products were analyzed by electrophoresis in 2.5 % agarose gel and stained with ethidium bromide or GelRed$^{TM}$. Presence or absence of *GSTM1* and *GSTT1* were observed by performing two multiplex PCRs. *NAT2* genotyping method was described by Possuelo *et al.* (Possuelo et al. 2008).

*GSTT1* amplification was performed using two different primers due to the frequency of gene deletion that was unexpectedly lower than that usually observed in other populations. For the second analysis, primers designed by other authors were used (Leiro et al. 2008). *GST*s were considered present when the fragment was amplified together

**TABLE I**
**Description of all Anti Tuberculisis Drug-induced hepatitis cases.**

| Case | Sex | Time (days)[a] | Age (years) | Bilirubin peak (mg/dL) | AST/ALT Peak (U/L) | Risk factors[b] |
|---|---|---|---|---|---|---|
| 1 | F | 13 | 27 | 0.60 | 148/705 | 1 |
| 2 | M | 15 | 66 | 1.5 | 857/548 | - |
| 3 | F | 17 | 38 | 0.72 | 233/429 | 1, 3, 5 |
| 4 | M | 14 | 31 | 1.05 | 275/503 | 2, 3,5 |
| 5 | F | 11 | 42 | 4.56 | 75/318 | 1 |
| 6 | F | 11 | 49 | 3.72 | 96/398 | 1, 3, 4 |
| 7 | M | 14 | 44 | 1.09 | 62/309 | 3 |
| 8 | M | 13 | 37 | 1.6 | 157/197 | 3, 4 |
| 9 | F | 14 | 19 | 1.35 | 134/406 | 1 |
| 10 | M | 8 | 30 | 1.6 | 153/254 | 3, 4 |
| 11 | F | 8 | 51 | 0.82 | 316/392 | 1, 2 |
| 12 | M | 17 | 46 | 0.61 | 234/804 | 3 |
| 13 | M | 13 | 25 | 0.42 | 339/188 | |
| 14 | F | 90 | 25 | 1.61 | 221/492 | 1, 3 |
| 15 | F | 5 | 27 | 0.69 | 93/85 | 1 |

[a] Time until diagnosis of hepatotoxicity. [b] Risk factors: 1) female sex, 2) Hepatitis C Virus, 3) Human immunodeficiency virus (HIV), 4) Highly active antiretroviral therapy (HAART), 5) alcohol abuse. F= female; M= male; AST= aspartate transaminase; ALT= alanine transaminase; Peak= highest level during treatment.

with the 560 bp *NAT2* DNA fragment, used as an internal control. When only the *NAT2* fragment was detected in the gel, *GST* was considered as a deleted gene.

### Statistical Analyses

Allele frequencies at individual SNPs were estimated by means of counting. Linkage disequilibrium (*D*) and *D'* (the relative magnitude of D as compared to its theoretical maximum) were also calculated using the software program MULTIPLE LOCUS HAPLOTYPE ANALYSIS release 2.0 (Multiple Locus Haplotype Analysis 1999, Long et al.1995).

All statistical analyses were performed using the SPSS statistical program, release 12.0 (SPSS, Chicago, IL). Values are expressed as means ± standard deviation (SD) or as numbers and percentages. Group comparisons for categorical variables were carried out using the $\chi^2$ test, while Student's *t* test was used for the analysis of continuous variables. Relative risk (RR) and confidence intervals (CI=95%) were calculated. Multiple logistic regression analyses were carried out using the backward model. All statistical tests were based on two-tailed probability and a p value ≤0.05 was considered significant. The multivariate model was generated using variables with a *p* value<0.20.

## RESULTS

### Patients Characteristics

Fifteen patients (6.1%) developed ATD-induced hepatitis (Table I). All of them have had their treatment switched to *streptomycin (SM)*, INH and EMB, an alternative treatment to RMP, INH and PZAHZ used in Rio Grande do Sul in the presence of ATD-induced hepatitis. None of the patients had a new episode of hepatotoxicity with the new regimen. Co-medication during TB treatment was used by 100 patients (40.8%). Of the 64 HIV-positive patients, 33 (51%) used co-medication and 3 of them developed hepatotoxicity (9%). ADR during treatment was observed in 48 (19.6%) patients. In the present study, only the mean AST and ALT values at baseline differed considerably between patients with and without ATD-induced hepatitis (Table II).

### Genotyping Analysis

Regarding the polymorphisms in CYP2E1 at position -1053, 210 (85.7%) patients were genotyped as *1A/*1A (wild-type), 31 (12.7%) *1A/*5B (heterozygous) and 4 (1.6%) *5B/*5B (mutant). At position -1293, 208 (84.9%) were *1A/*1A (wild-type), 32 (13.1%) *1A/*5B (heterozygous) and 5 (2%) *5B/*5B (mutant). For polymorphism in intron 6, 191 (78%) patients were genotyped as *1A/*1A (wild-type), 49 (20%) *1A/*6 (heterozygous) and 5 (2%) *6/*6 (mutant). The *GSTM1* null mutation was found in 105 patients (42.9%) and *GSTT1* null genotype in 30 patients (12.2%). The most frequent NAT2 genotypes were *5/12 (31.3%), *12/12 (15%), *5/5(12.2%), 6/6 (8.1%) and 4/4 (6.1%). The results of a linkage disequilibrium test carried out with *CYP2E1* SNPs are shown in Supplemantary Table II.

**TABLE II**
**Univarite analysis of clinical risk factors for ATD- induced hepatitis.**

| | Hepatotoxicity | | Total | *p* |
|---|---|---|---|---|
| | Yes | No | | |
| Mean Age (± SD) | 38.1 (±12.79) | 36.8 (±12.8) | 36.8 (±12.8) | 0.7 |
| BMI ($kg/m^2$) | 21.3 (±3.1) | 20.7 (±3.3) | 20.7 (± 3.2) | 0.2 |
| AST (U/L) baseline | 41.9 (±36.2) | 26.5 (±16) | 28.1 (±20.3) | 0.007 |
| ALT (U/L) baseline | 40.5 (±32.6) | 28,6 (±19.1) | 29.8 (±20.1) | 0.04 |
| Total bilirubin (mg/dL) baseline | 0.5 (±0.18) | 0.4 (±0.28) | 0.4 (±0.27) | 0.6 |
| AST (U/L) peak | 187.9 (±89.3) | 37.8 (± 78.9) | 52.6 (±91.4) | <0.001 |
| ALT (U/L) peak | 419.8 (±182.9) | 43.5 (±54.8) | 80.5 (±135.8) | <0.001 |
| Total bilirubin (mg/dL) peak | 1.2 (±0.9) | 0.4 (±0.6) | 0.47 (±0.7) | <0.001 |

SD – stand deviation; BMI – body mass index; AST- Aspartate transaminase; ALT- Alanine transaminase; peak- the highest level after commencing the treatment.

### Association Among Clinical Factors, Genotypes and ATD-Induced Hepatitis

Of the 15 patients with ATD-induced hepatitis, none were homozygous mutant for *CYP2E1* and only one had both *GST* genes deleted. In these patients, no difference was observed regarding polymorphism frequencies in relation to all patients.

Combined analysis of the polymorphisms in *CYP2E1, GSTM1* and *GSTT1* genes was performed, but no synergistic effect was observed. When the *NAT*2 slow phenotype was combined with *CYP2E1 Rsa*I *1A/*1A genotype, the association with hepatotoxicity was stronger (Table III).

Analysis of liver function tests before the start of the treatment showed that 13% of patients had AST levels higher than the ULN, 6.2% had altered AST levels and 1.2% had bilirubin alteration. Among patients with altered AST levels before treatment, 25% were 3-fold or more above the normal limit. Comparative analysis using mean baseline ALT and AST and GSTM1, CYP2E1, GSTT1 and NAT2 genotypes was performed and no significant differences were observed. AST, ALT and total bilirubin levels after 30 days of treatment were significantly higher in patients homozygous (wild type) for the *Rsa*I *1A allele ($p<0.05$).

After performing multivariate analysis, HIV, female sex, hepatotoxicity and NAT2 slow acetylator were significantly associated with hepatotoxicity in this population (Table IV).

## DISCUSSION

Our study shows the frequency of polymorphisms in *CYP2E1, GSTM1* and *GSTT1*, their association with ATD-induced hepatitis and clinical risk factors. The main finding in the present study is that the association between genotype and hepatotoxicity is only observed when it includes NAT2 slow acetylator. In a study carried out in Brazil, the *NAT2, CYP2E1, GSTM1* and *GSTT1* polymorphisms were genotyped with regimens containing INH, where slow acetylators had a higher incidence of ATD-induced hepatitis than intermediate acetylators. The logistic regression showed that slow acetylation was a risk factor for drug-induced hepatotoxicity (OR 3.59, 95% CI, 2.53-4.64, p = 0.02) (Teixeira

**TABLE III**
**Genotype and allelic characterization of the *CYP*2E1, *GST*M1, *GST*T1 and acetylation profile of NAT2 in tuberculosis patients with and without hepatotoxicity.**

| | Hepatotoxicity | | Total | p | OR (95%IC) |
|---|---|---|---|---|---|
| | Yes N=15 (%) | No N=230 (%) | | | |
| ***CYP*2E1 *Pst*I** | | | | | |
| ***1A/1A** | 13 (86.7) | 195 (84.8) | 208 ( 84.9) | 1.0 | 0.85 (0.18-3.56) |
| ***1A/*5B + *5B/5B** | 2 (13.3) | 35 (15.2) | 37 (15.1) | | |
| **1A[a]** | 28 (6.2)[b] | 420 (93.8)[c] | 448 (91.4)[d] | | |
| **5B[a]** | 2(4.8)[b] | 40 (95.2)[c] | 42 (8.6)[d] | | |
| ***CYP*2E1 *Rsa*I** | | | | | |
| ***1A/1A** | 14 (93.3) | 196 (85.2) | 210 (85.7) | 0.7 | 0.41 (0.05-3.23) |
| ***1A/*5B + *5B/5B** | 1 (6.7) | 34 (14.8) | 35 (14.3) | | |
| **1A[a]** | 29(7.0)[b] | 422()[c] | 451(92.0)[d] | | |
| **5B[a]** | 1(2.6)[b] | 38(97.4)[c] | 39(8.0)[d] | | |
| ***CYP*2E1 *Dra*I** | | | | | |
| ***1A/1A** | 12 (80) | 179 (77,8) | 191 (78) | 1.0 | 0.83 (0.22-3.06) |
| ***1A/*6 + *6/6** | 3(20) | 51 (22.2) | 54 (22) | | |
| **1A[a]** | 27(6.3)[b] | 404(93.7)[c] | 431(88.0)[d] | | |
| **6[a]** | 3(5.1)[b] | 56(94.9)[c] | 59(22.0)[d] | | |
| ***GST*M1 null** | 6 (40) | 99 (43) | 105 (42.9) | 1.0 | 0.88 (0.30-2.56) |
| ***GST*T1 null** | 2 (13.3) | 28 (12.2) | 30 (12.4) | 1.0 | 1.1 (0.23-5.17) |
| ***GST*M1 + *GST*T1 null** | 1 (6.7) | 12 (5.2) | 13 (5.3) | 0.57 | 1.3 (0.15-10.7) |
| ***NAT*2 slow** | 9 (60) | 56 (24.3) | 65 (26.5) | 0.03 | 5.46 (1.75-16.98) |
| ***NAT*2 slow + *GST*M1null** | 2 (13.3) | 46 (20) | 48 (19.6) | 0.7 | 0.6 (0.13-2.8) |
| ***NAT*2 slow + *CYP Rsa*I*1A** | 9 (60) | 49(21.3) | 58(23.7) | 0.002 | 5.5 (1.88-16.3) |
| ***NAT*2 slow + *CYP Rsa*I*1A + *CYP Pst*I*1A + *CYP Dra*I*1A** | 7 (46.7) | 43 (18.7) | 50 (20.4) | 0.017 | 3.8 (3.3-11.06) |

[a]Allelic frequency; [b] Frequency refers to 460 alleles; [c] Frequency refers to 30 alleles; [d] Frequency refers to 490 alleles; OR – odds ratio; CI- confidence interval; NAT2- N-acetyltransferase 2; GSTT1- glutathione *S*-transferase T1; GSTM1- glutathione *S*-transferase M1; CYP- cytochrome P450.

et al. 2011). *NAT2* is one of the factors responsible for genetic predisposition to INH-induced hepatitis and *NAT2* acetylating status is related to the patient's condition, sex and ethnic origin, so it may be regarded as important risk factor for the development of hepatotoxicity (Mahmoud et al. 2012, Chamorro et al. 2013).

Many studies reported the two polymorphisms in the 5' flanking region of *CYP2E1* as being in complete linkage disequilibrium (LD). In our study, similar to the results described by Kato et al (1992), *Rsa*I and *Pst*I polymorphisms were not in total LD. Five patients did not have those polymorphisms linked in LD: 3 were *Rsa*I *1A/*1A and *Pst*I *1A/*5B; one was *Rsa*I *1A/*5B and *Pst*I *1A/*1A and one was *Rsa*I *1A/*5B and *Pst*I *5B/*5B. Earlier reports showed that the mutant allele *5B was associated with higher transcriptional activity, protein levels and enzyme activity than the common wild type allele *1A (Hayashi et al. 1991, Watanabe et al. 1994). However, opposing data have been reported in more recent studies (An et al. 2012, Huang et al. 2003). In the population studied by Huang and cols, wild-type patients (*1A/*1A) were at increased risk for ATD-induced hepatitis (Huang et al. 2003). Vuilleumier et al. reported a significant association between the wild-type *CYP2E1* and elevated liver enzymes. Nevertheless they did not observe a significant association between INH-induced hepatitis and *CYP2E1* genotypes (Vuilleumier et al. 2006). The *CYP2E1* wild-type

**TABLE IV**
**Multivariate logistic analysis of risk factors associated with the development of hepatotoxicity.**

| Characteristic | Bivariate analysis: odds ratio (95% CI) | *p* | Multivariate analysis: odds ratio (95% CI) | *p* |
|---|---|---|---|---|
| HCV positive | 0.71 (0.15-3.3) | 0.5 | ------- | |
| Alcohol use | 0.8 (0.17-3.7) | 1.0 | ------- | |
| White skin color | 0.5 (0.14- 1.5) | 0.3 | ------- | |
| Co-medication | 0.96 (0.33-2.80) | 1.0 | ------- | |
| AST baseline | 3.4 (0.99-12.07) | 0.06 | 0.39 (0.01-11.28) | 0.58 |
| ALT baseline | 3.02 0.60-17.07 | 0.18 | 18.04 (0.50-640.0) | 0.11 |
| HBV positive | 5.4 (0.52-55.6) | 0.2 | 2.12 (0.18-247.8) | 0.75 |
| HAART | 3.13 (1.6-13.7) | 0.1 | 1.64 (0.25-10.65) | 0.60 |
| HIV positive | 3.5 (1.23-10.2) | 0.03 | 6.46 (1.20-36.68) | 0.01 |
| Female | 0.43 (0.15-1.23) | 0.2 | 5.9 (1.22- 28.76) | 0.02 |
| Extrapulmonary TB | 3.8 (1.3 -11.5) | 0.02 | 5.1 (1.09 23.83) | 0.03 |
| Slow acetylator profile – NAT2 | 5.46 (1.75-16.98) | 0.03 | 14.59 (2.92-72.74) | 0.001 |

HAART- Highly active antiretroviral therapy; HIV- Human immunodeficiency virus; HC(B)V-Hepatitis C(B) virus; CI- Confidence interval; AST- Aspartate transaminase; ALT- Alanine transaminase; OR-odds ratio; CI- confidence interval.

genotype is often associated with high enzyme activity and high production of hepatotoxins (Roy et al. 2008, Tostmann et al. 2008), which corroborates our results, where patients with *Rsa*I wild-type had higher liver enzyme levels after start of treatment. In a study with rats, administration of INH increased plasma concentrations of hydrazine and *CYP2E1* activity, sometimes leading to hepatotoxicity (Yue et al. 2004). In another study with rats, the results suggested that *CYP2E1* null is not involved with hepatotoxicity when INH is prescribed. (Cheng et al. 2013). In agreement with the study performed by Cho (Cho et al. 2007), we found no direct association between *CYP2E1* polymorphisms and hepatotoxicity.

The *CYP2E1* frequencies observed in our study are more similar to those found in European-Americans of European descent (92% *1A/*1A, 7% *1A/*5B and 1% *5B/*5B for *Rsa*I/*Pst*I; 10% *1A and 90% *6 for *Dra*I) (Roy et al. 2008). Polymorphisms in *CYP2E1* are present at higher frequencies in Asians than any other populations (Garte et al. 2001), with a frequency of about 20-30% of the *5B allele (Khan et al. 2009). According to the meta-analysis study by Cai (Cai et al. 2012) the *NAT2* slow genotypes, *CYP2E1**1A and *GSTM1* null have a modest effect on the genetic susceptibility to hepatotoxicity. Another study reported that the administration of the flavonoid quercetin could neutralize the hepatotoxicity mediator *CYP2E1* (Tang et al., 2013).

*GSTs* are a superfamily of enzymes involved in a range of biological detoxification processes. Whole *GSTM1* and *GSTT1* genes show high percentage of deletion in the human population (Bolt and Thier, 2006) and this is expected to contribute to interindividual differences in response to xenobiotics (Hayes et al. 2005). *GSTM1* and *GSTT1* deletions are the best choices for drug-induced liver injury in association studies (Roy et al. 2001, Hussain et al. 2003, Leiro et al. 2008, Huang, 2010).

In our study, *GSTM1* was deleted in almost 43% of the population, comparable to that observed in studies with Hispanic and European-American populations (Leiro et al. 2008, Roy et al. 2008). *GSTT1* was deleted in 12.4% of the present population, a lower frequency than the one commonly described in European-American and Brazilian populations (Gaspar et al. 2004, Kvitko

et al. 2006, Roy et al. 2008, Magno et al. 2009) , but similar to Hispanic and Scandinavian populations (Garte et al. 2001, Roy et al. 2008). Data showed some associations between *GSTT1* (Leiro et al. 2008) and *GSTM1* (Roy et al. 2001, Huang et al. 2007) null genotypes and hepatotoxicity. The *GSTM1* and *GSTT1* may play a role in the susceptibility to drug-induced development of liver injury, which occurs regardless of the type of drug used, being more prevalent in women (Lucena et al. 2008). In our study no such association was observed. A study carried out in São Paulo (SP-Brazil) and Maringá (PR-Brazil) concluded that in addition to *NAT2* polymorphisms, *CYP2E1* and *GSTM1/ GSTT1* the use of *CYP2E1* inhibitors contribute to susceptibility to mild alterations in liver enzymes in patients receiving anti-tuberculosis drug therapy (Forestiero et al. 2013).

Based on the study by Monteiro et al. (2012) *GSTM1* null genotype and *GSTT1* do not appear to play a major role in hepatic injury induced by anti-tuberculosis drugs in Brazil, although there has been evidence that *GSTM1* is associated to toxicity intensity. As for the genotyping of *GSTM1* and *GSTT1* polymorphisms, a case-control and one Indian population found that both null genotypes seem to be associated with drug-induced hepatotoxicity (Chatterjee et al. 2010). It is widely known that Brazil has one of the most genetically-mixed populations in the world. However the southernmost state, Rio Grande do Sul, has had a singular colonization. Portuguese people from the Azores were the very first immigrants to arrive in the capital city, Porto Alegre, followed by immigrants from Spain, Italy and Germany. The gene pool also has the contribution of African slaves and Amerindians. This mix is hardly exclusive to the South of the country and genetics differences have been established by studies using ancestry informative markers (Callegari-Jacques et al. 2003, Marrero et al. 2007, Guerreiro-Junior et al. 2009).

The incidence of ATD-induced hepatitis in our study was 6.1%. This incidence rate varies extensively in different populations, for instance, 9.7% in Malaysia (Marzuki et al. 2008), 11.9% in Spain (Fernández-Villar et al. 2004) and 3% in Canada (Yee et al. 2003). After the joint analysis including genetic and clinical risk factors for ATD-induced hepatitis it was observed that HIV, female sex, extrapulmonary TB and NAT2 slow acetylator profile are risk factors in our population. Other risk factors were observed in other populations, such as female sex, older age and pre-existing liver diseases (B and C hepatitis) (Saukkonen et al. 2006), but in our study no association was observed with these factors. The recommendation to design a study excluding patients with liver injuries, in order to have a clear understanding of the anti-TB drug effect on the liver was not possible at present, due to the high number of affected patients.

In conclusion, our study indicated that *NAT2* slow acetylator profile, HIV, extrapulmonary tuberculosis and female sex predispose an individual to ATD-induced hepatitis. Further studies with a higher number of hepatotoxicity cases and simultaneous analysis of more polymorphisms should be carried out in different ethnic populations and in different regions of Brazil.

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**RESUMO**

Durante o tratamento para tuberculose (TB) um dos efeitos adversos mais graves é a hepatite induzida por drogas (ATD) que tem sido associada a mutações nos genes que codificam as enzimas metabolizadoras destas drogas.

A terapia de seis meses é composta por isoniazida, (INH), rifampicina (RMP), pirazinamida (PZA) e etambutol (EMB). N-acetiltransferase 2 *(NAT2)*, citocromo P450 2E1 (*CYP2E1)* e glutationa-S-transferase *(loci GSTM1 e GSTT1)* estão envolvidos no metabolismo da isoniazida, o fármaco mais tóxico no tratamento da TB. Este estudo foi desenhado para estimar a frequência dos polimorfismos nos genes *CYP2E1, GSTM1* e *GSTT1* que estão relacionados com a resposta à essas drogas, e também identificar fatores clínicos de risco para ATD. Foram incluídos no estudo 245 pacientes brasileiros em tratamento para TB que foram genotipados utilizando a reação em cadeia de polímeras e sequenciamento dos polimorfismos. As frequências de alelos polimórficos *CYP2E1 Rsal, Dral e PstI* encontradas forame 8%, 8,5% e 12%, respectivamente. Os genes *GSTM1* e *GSTT1* estão ausentes em 42,9% e 12,4% da população, respectivamente. Quinze pacientes (6,1%) desenvolveram hepatotoxicidade. As características clínicas (HIV, sexo feminino e TB extrapulmonar) e *NAT2* perfil de acetilação lenta estão em maior risco de ATD nesta população. Genótipo para *GSTM1* e *GSTT1* não mostrou nenhuma influência na resposta à droga.

**Palavras-chave**: Hepatotoxicidade, isoniazida, polimorfismos, tuberculose.

## REFERENCES

An HR, Wu XQ, Wang ZY, Zhang JX and Liang Y. 2012. NAT2 and CYP2E1 polymorphisms associated with antituberculosis drug-induced hepatotoxicity in Chinese patients. Clin Exp Pharmacol Physiol 39: 535-543.

Arif E, Vibhuti A, Alam P, Deepak D, Singh B, Athar M and Pasha MA. 2007. Association of CYP2E1 and NAT2 gene polymorphisms with chronic obstructive pulmonary disease. Clin Chim Acta 382: 37-42.

Bénichou C. 1990. Criteria of drug-induced liver disorders. Report of an international consensus meeting. J Hepatol 11: 272-276.

Boccia S, De Lauretis A, Gianfagna F, Van Duijn CM and Ricciardi G. 2007. CYP2E1PstI/RsaI polymorphism and interaction with tobacco, alcohol and GSTs in gastric cancer susceptibility: A meta-analysis of the literature. Carcinogenesis 28: 101-106.

Bolt H and Thier R. 2006. Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr Drug Metab 7: 613-628.

Cai Y, Yi J, Zhou C and Shen X. 2012. Pharmacogenetic Study of Drug-Metabolising Enzyme Polymorphisms on the Risk of Anti-Tuberculosis Drug-Induced Liver Injury: A Meta-Analysis. PLoS One 7: e47769.

Callegari-Jacques SM, Grattapaglia D, Salzano FM, Salamoni SP, Crossetti SG, Ferreira ME and Hutz MH. 2003. Historical genetics: spatiotemporal analysis of the formation of the Brazilian population. Am J Hum Biol 15: 824-834.

Castelo Filho A et al. 2004. II Consenso Brasileiro de Tuberculose: Diretrizes Brasileiras para Tuberculose 2004. J Bras de Pneumol 30: s57-s86.

Chamorro JG, Castagnino JP, Musella RM, Nogueras M, Aranda FM, Frías A, Visca M, Aidar O, Perés S and De Larrañaga GF. 2013. Sex, ethnicity, and slow acetylator profile are the major causes of hepatotoxicity induced by antituberculosis drugs. J Gastroenterol Hepatol 28: 323-328.

Chatterjee S, Lyle N, Mandal A and Kundu S. 2010. *GSTT1* and *GSTM1* gene deletions are not associated with hepatotoxicity caused by antitubercular drugs. J Clin Pharm Ther 35: 465-470.

Cheng J, Krausz KW, Li F, Ma X and Gonzalez FJ. 2013. CYP2E1-dependent elevation of serum cholesterol, triglycerides, and hepatic bile acids by isoniazid. Toxicol Appl Pharmacol 266: 245-253.

Cho HJ, Koh WJ, Ryu YJ, Ki CS, Nam MH, Kim JW and Lee SY. 2007. Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis. Tuberculosis (Edinb) 87: 551-556.

Conde M et al. 2009. III Brazilian Thoracic Association Guidelines on tuberculosis. J Bras Pneumol 35: 1018-1048.

Eichelbaum M, Ingelman-Sundberg M and Evans WE. 2006. Pharmacogenomics and individualized drug therapy. Annu Rev Med 57: 119-137.

Fernández-Villar A, Sopeña B, Fernández-Villar J, Vázquez-Gallardo R, Ulloa F, Leiro V, Mosteiro M and Piñeiro L. 2004. The influence of risk factors on the severity of anti-tuberculosis drug-induced hepatotoxicity. Int J Tuberc Lung Dis 8: 1499-1505.

Forestiero FJ, Cecon L, Hirata MH, de Melo FF, Cardoso RF, Cerda A and Hirata RD. 2013. Relationship of NAT2, CYP2E1 and GSTM1/GSTT1 polymorphisms with mild elevation of liver enzymes in Brazilian individuals under anti-tuberculosis drug therapy. Clin Chim Acta 415: 215-219.

Garte S et al. 2001. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 10: 1239-1248.

Gaspar P, Moreira J, Kvitko K, Torres M, Moreira A and Weimer T. 2004. CYP1A1, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, and *TP53* polymorphisms: do they indicate susceptibility to chronic obstructive pulmonary disease and non-small-cell lung cancer? Genet Mol Biol 27:133-138.

GUERREIRO-JUNIOR V, BISSO-MACHADO R, MARRERO A, HÜNEMEIER T, SALZANO F AND BORTOLINI MC. 2009. Genetic Signatures of parental contribution in black and white populations in Brazil. Genet Mol Biol 32: 1-11.

HAYASHI S, WATANABE J AND KAWAJIRI K. 1991. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome *P450IIE1* gene. J Biochem 110: 559-565.

HAYES JD, FLANAGAN JU AND JOWSEY IR. 2005. Glutathione transferases. Annu Rev Pharmacol Toxicol 45: 51-88.

HUANG YS. 2010. Tailored drug therapy for mitigating drug-innduced liver injury: is this the era of geetic screening? Personalized Medicine 7: 5-8.

HUANG YS, CHERN HD, SU WJ, JC WU, CHANG SC, CHIANG CH, CHANG FY AND LEE SD. 2003. Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. Hepatology 37: 924-930.

HUANG YS, SU WJ, HUANG YH, CHEN CY, CHANG FY, LIN HC AND LEE SD. 2007. Genetic polymorphisms of manganese superoxide dismutase, NAD (P) H: quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. J Hepatol 47: 128-134.

HUSSAIN Z, KAR P AND HUSAIN SA. 2003. Antituberculosis drug-induced hepatitis: risk factors, prevention and management. Indian J Exp Biol 41: 1226-1232.

INGELMAN-SUNDBERG M. 2004. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. Naunyn Schmiedebergs Arch Pharmacol 369: 89-104.

INGELMAN-SUNDBERG M, SIM SC, GOMEZ A AND RODRIGUEZ-ANTONA C. 2007. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 116: 496-526.

KATO S, SHIELDS PG, CAPORASO NE, HOOVER RN, TRUMP BF, SUGIMURA H, WESTON A AND HARRIS CC. 1992. Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res 52: 6712-6715.

KHAN AJ, RUWALI M, CHOUDHURI G, MATHUR N, HUSAIN Q AND PARMAR D. 2009. Polymorphism in cytochrome *P4502E1* and interaction with other genetic risk factors and susceptibility to alcoholic liver cirrhosis. Mutat Res 664: 55-63.

KVITKO K, GASPAR PDA, TORRES M AND HUTZ M. 2006. *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in an Afro-Brazilian group. Genet Mol Biol 29: 613-616.

LEIRO V, FERNÁNDEZ-VILLAR A, VALVERDE D, CONSTENLA L, VÁZQUEZ R, PIÑEIRO L AND GONZÁLEZ-QUINTELA A. 2008. Influence of glutathione S-transferase M1 and T1 homozygous null mutations on the risk of antituberculosis drug-induced hepatotoxicity in a Caucasian population. Liver Int 28: 835-839.

LIU R, YIN LH AND PU YP. 2007. Association of combined CYP2E1 gene polymorphism with the risk for esophageal squamous cell carcinoma in Huai'an population, China. Chin Med J (Engl) 120: 1797-1802.

LONG JC, WILLIAMS RC AND URBANEK M. 1995. An E-M algorithm and testing strategy for multiple-locus haplotypes. Am J Hum Genet 56: 799-810.

LUCENA MI ET AL. 2008. Glutathione S-Transferase M1 and T1 Null Genotypes Increase Susceptibility to Idiosyncratic Drug-Induced Liver Injury. Hepatology 48: 588-596.

MAGNO LA ET AL. 2009. Glutathione s-transferase variants in a brazilian population. Pharmacology 83: 231-236.

MAHMOUD BG, GHOZZI H, KAMOUN A, HAKIM A, HACHICHA H, HAMMAMI S, SAHNOUN Z, ZALILA N, MAKNI H AND ZEGHAL K. 2012. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatotoxicity in Tunisian patients with tuberculosis. Pathol Biol 60: 324-330.

MARRERO AR ET AL. 2007. Pre- and post-Columbian gene and cultural continuity: the case of the Gaucho from southern Brazil. Hum Hered 64: 160-171.

MARZUKI OA, FAUZI AR, AYOUB S AND KAMARUL IMRAN M. 2008. Prevalence and risk factors of anti-tuberculosis drug-induced hepatitis in Malaysia. Singapore Med J 49: 688-693.

MAYFIELD D, MCLEOD G AND HALL P. 1974. The CAGE questionnaire: validation of a new alcoholism screening instrument. Am J Psychiatry 131: 1121-1123.

MILLER SA, DYKES DD AND POLESKY HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.

MONTEIRO TP, EL-JAICK KB, JEOVANIO-SILVA AL, BRASIL PE, COSTA MJ, ROLLA VC AND CASTRO L. 2012. The roles of *GSTM1* and *GSTT1* null genotypes and other predictors in anti-tuberculosis drug-induced liver injury. J Clin Pharm Ther 37: 712-718.

MULTIPLE LOCUS HAPLOTYPE ANALYSIS. 1999. Computer program, Version 2.0. Bethesda: Laboratory of Neurogenetics (NIAAA), National Institute of Health.

POSSUELO LG ET AL. 2008. Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil. Eur J Clin Pharmacol 64: 673-681.

ROY B, CHOWDHURY A, KUNDU S, SANTRA A, DEY B, CHAKRABORTY M AND MAJUMDER P. 2001. Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. J Gastroenterol Hepatol 16: 1033-1037.

ROY PD, MAJUMDER M AND ROY B. 2008. Pharmacogenomics of anti-TB drugs-related hepatotoxicity. Pharmacogenomics 9: 311-321.

SAUKKONEN JJ ET AL. 2006. An official ATS statement: hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med 174: 935-952.

STINE JG, SATEESH P AND LEWIS JH. 2013. Drug-Induced Liver Injury in the Elderly. Curr Gastroenterol Rep 15: 299.

SUAREZ-KURTZ G AND PENA SD. 2006. Pharmacogenomics in the Americas: the impact of genetic admixture. Curr Drug Targets 7: 1649-1658.

TANG Y, TIAN H, SHI Y, GAO C, XING M, YANG W, BAO W, WANG D, LIU L AND YAO P. 2013. Quercetin suppressed *CYP2E1*-dependent ethanol hepatotoxicity via depleting heme pool and releasing CO. Phytomedicine 20: 699-704.

TEIXEIRA RL, MORATO RG, CABELLO PH, MUNIZ LM, MOREIRA ADA S, KRITSKI AL, MELLO FC, SUFFYS PN, MIRANDA AB AND SANTOS AR. 2011. Genetic polymorphisms of *NAT2*, *CYP2E1* and *GST* enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. Mem Inst Oswaldo Cruz 106: 716-724.

TOSTMANN A, BOEREE MJ, AARNOUTSE RE, DE LANGE WC, VAN DER VEN AJ AND DEKHUIJZEN R. 2008. Antitubeculosisr drug-induced hepatotoxicity: concise up-to-date review. J Gastroenterol Hepatol 23: 192-202.

VUILLEUMIER N ET AL. 2006. CYP2E1 genotype and isoniazid-induced hepatotoxicity in patients treated for latent tuberculosis. Eur J Clin Pharmacol 62: 423-429.

WALKER NF, KLINER M, TURNER D, BHAGANI S, CROPLEY I, HOPKINS S AND LIPMAN M. 2009. Hepatotoxicity and antituberculosis therapy: time to revise UK guidance? Thorax 64: 918.

WATANABE J, HAYASHI S AND KAWAJIRI K. 1994. Different regulation and expression of the human CYP2E1 gene due to the RsaI polymorphism in the 5'-flanking region. J Biochem 116: 321-326.

YEE D, VALIQUETTE C, PELLETIER M, PARISIEN I, ROCHER I AND MENZIES D. 2003. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. Am J Respir Crit Care Med 167(11): 1472-1477.

YEW WW AND LEUNG CC. 2006. Antituberculosis drugs and hepatotoxicity. Respirology 11: 699-707.

YIMER G ET AL. 2011. Pharmacogenetic & Pharmacokinetic Biomarker for Efavirenz Based ARV and Rifampicin Based Anti-TB Drug Induced Liver Injury in TB-HIV Infected Patients. PLoS One 6: e27810.

YUE J, PENG RX, YANG J, KONG R AND LIU J. 2004. *CYP2E1* mediated isoniazid-induced hepatotoxicity in rats. Acta Pharmacol Sin 25: 699-704.