

# Polymorphisms in the *CYP2E1* and *GSTM1* Genes as Possible Protection Factors for Leprosy Patients

Pablo Pinto<sup>1</sup>, Claudio Guedes Salgado<sup>2</sup>, Ney Santos<sup>1</sup>, Dayse O. Alencar<sup>1</sup>, Sidney Santos<sup>1</sup>, Mara H. Hutz<sup>3</sup>, Ândrea Ribeiro-dos-Santos<sup>1\*</sup>

**1** Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brasil, **2** Laboratório de Dermatoinmunologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brasil, **3** Instituto de Biociências, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brasil

## Abstract

**Background:** The *CYP2E1* and *GSTM1* genes encode metabolic enzymes that have key functions in drug modification and elimination.

**Methodology/Principal Findings:** We investigated the possible effects of *CYP2E1* and *GSTM1* polymorphisms in 71 leprosy patients and in 110 individuals from the general population. The *GSTM1\*0* null allele and INDEL *CYP2E1\*1D* mutant genotypes were analyzed by conventional PCR, while *CYP2E1* SNPs (1053C>T, 1293G>C and 7632T>A) were determined by RT-PCR. In leprosy patients, the *GSTM1\*0* and *CYP2E1\*5* alleles and the combined alleles *GSTM1\*0/CYP2E1\*6* and *GSTM1\*0/CYP2E1\*5* were significantly related to a bacilloscopic index (BI) (BI<3), while the *CYP2E1\*6* allele was related to a better clinical evolution in the leprosy spectrum.

**Conclusions/Significance:** Therefore, *GSTM1\*0*, *CYP2E1\*5* and *CYP2E1\*6* may be possible protection factors for leprosy patients.

**Citation:** Pinto P, Salgado CG, Santos N, Alencar DO, Santos S, et al. (2012) Polymorphisms in the *CYP2E1* and *GSTM1* Genes as Possible Protection Factors for Leprosy Patients. PLoS ONE 7(10): e47498. doi:10.1371/journal.pone.0047498

**Editor:** Tanya Parish, Queen Mary University of London, United Kingdom

**Received:** April 3, 2012; **Accepted:** September 17, 2012; **Published:** October 15, 2012

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**Funding:** This study was supported by FINEP (Financiadora de Estudos e Projetos), CNPq/Casadinho (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento Pessoal de Nível Superior) and PROPES/UFPA (Universidade Federal do Pará), FADESP (Fundação de Amparo a Pesquisa). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: akely@ufpa.br

## Introduction

Leprosy is an insidious infectious disease caused by the obligate intracellular bacteria *Mycobacterium leprae* that affects the skin and peripheral nerves, causing a chronic granulomatous infection [1].

Multidrug therapy (MDT), the treatment recommended by the World Health Organization (WHO), has healed millions of patients since it was implemented in 1980s. MDT consists of the use of dapsone and rifampicin for 6 months in paucibacillary (PB) patients, or both along with a third drug, clofazimine, for 12 months in multibacillary (MB) cases [2].

Patients are classified as PB or MB using a simple system introduced by the WHO in 1982. Patients with 5 skin lesions or less are classified as PB, and those with more than 5 lesions are classified as MB [3,4]. Although this simple classification scheme is adequate for remote sites where the population has little or no access to health care, it is not detailed enough for more in-depth research surveys.

Another way to classify leprosy patients is based on a skin smear test, for which a positive result is classified as MB, and a negative result as PB. A trained laboratory technician can readily identify AFB (acid fast bacillus), making this test a very reliable method. However, cases initially classified as PB (AFB negative) can evolve to MB in the natural course of the disease [5]. This phenomenon is

especially true for those patients classified as indeterminate (MHI) using the Madrid classification system.

The use of the Ridley-Jopling clinical, histological and immunological criteria further improves case definitions, with TT (tuberculoid-tuberculoid) patients exhibiting a strong cellular immune response (CIR) and a negative skin smear test, while LL (lepromatous-lepromatous) patients have a weak or absent CIR and a highly positive skin smear [6]. In the middle of the spectrum are a large number of borderline patients, varying from weak to strong CIR and from negative to positive skin smears.

Interestingly, neither the CIR status nor the skin smear test is predictive of leprosy reactions or of the progression of each case through the physical disabilities caused by the disease. To date, little is known about which factors are crucial to the development of these disabilities. All types of patients, TT, borderline or LL, can progress with highly incapacitating disabilities and chronic neuropathies with no single marker or criteria to predict patient outcome [2,3].

Dapsone (4,4'-diaminodiphenyl sulfone, DDS) is one of the primary drugs used in anti-leprosy therapeutics. It is a bacteriostatic agent that competes with para-aminobenzoic acid (PABA), diminishing or blocking the production of bacterial folic acid [5,7]. Clofazimine is a riminophenazine dye that has bactericidal and anti-inflammatory effects. It inhibits bacterial proliferation by

binding to bacterial DNA and blocking its replication [8,9]. Rifampicin, or rifampin (RIF) has a well-proven bactericidal effect on *M. leprae*. It is a semisynthetic drug, originally derived from *Streptomyces mediterranei*, and it is widely used for treating leprosy and tuberculosis. RIF prevents protein production by inhibition of RNA polymerase in bacterial cells [10–16].

CYP-450 members of the heme protein superfamily are notable for their large spectrum of action and the distribution of their biological structures. These proteins participate in critical processes including the biosynthesis of steroidal hormones and the detoxification by conjugation with cellular components, such as glutathione [17].

CYP2E1 is found in various tissues including brain, lungs and kidneys, but it is most concentrated in the liver, where the majority of biotransformation occurs. Four main SNPs in the *CYP2E1* gene have been investigated in different populations, including tuberculosis (TB) patients [18–20], who often show 1053C>T and 1293G>C mutations, which together form the compound allele *CYP2E1\*1A*, CG; *CYP2E1\*5*, TC. Another SNP, 7632T>A, is located in the sixth intron of the *CYP2E1* gene and has two alleles, wild-type *CYP2E1\*1A* (T) and mutant *CYP2E1\*6* (A). Additionally, a 96-bp INDEL polymorphism with two alleles, wild-type *CYP2E1\*1C* (DEL) and mutant *CYP2E1\*1D* (INS), has also been described [18,19].

There are different glutathione S-transferase (GST) isoforms, including *GSTM1*, which is located on chromosome 1. More than 51 SNPs have been described within *GSTM1*, among which are two functional alleles, *GSTM1\*A* and *GSTM1\*B*, that have the same detoxification efficacy; one null (deletion) allele, *GSTM1\*0*; and one duplication [21,22].

Two *GSTM1* polymorphisms, *GSTM1\*1*, which has normal activity, and *GSTM1\*0*, which has no enzymatic activity because it is a complete gene deletion, have been well studied in different populations [23–25]. The presence of the null allele seems to be related to substrate conjugation and excretion; therefore, its presence can be an indicator for more rational drug dosages for various groups of patients [26].

We investigated a sample of MDT-treated leprosy patients ascertained at the Dr. Marcello Candia Reference Unit in the Sanitary Dermatology of the State of Pará (UREMC) with the aim of identifying associations among *CYP2E1* polymorphisms [including 1053 C>T, 1293G>C (*CYP2E1\*1A*, *CYP2E1\*5*); 7632T>A (*CYP2E1\*1A*, *CYP2E1\*6*); 96-bp INDEL *CYP2E1\*1C* (DEL) and *CYP2E1\*1D* (INS)] and *GSTM1* polymorphisms (*GSTM1\*1* and *GSTM1\*0*) and possible protection factors for leprosy patients.

## Methods

### Sample

We investigated 71 leprosy patients who attended the Dr. Marcello Candia Reference Unit in the Sanitary Dermatology of the State of Pará (UREMC) in Marituba, Pará, Brazil, from January 2008 to December 2009. In UREMC there are about 40,000 yearly consultations on different medicine specialties as, among others, leprology, dermatology, ophthalmology and orthopedics, besides nursery, physical therapy and other health professionals sessions. Since 2002, UREMC registered between 308 and 472 leprosy patients (mean: 408 cases/year). During years 2008 and 2009, 765 leprosy cases were registered, from those, 71 (9,28%) were randomly selected for this study.

All patients were evaluated neurologically by Semmes-Weinstein monofilament examination (SWME) for sensory testing, and by voluntary motor testing (VMT) for function assessment of

muscular force, as previously described [27]. They were classified according to the Ridley-Jopling system and were distributed in two groups depending on the progression of the disease: the positive (+) group consisted of PB patients skin smear negative Tuberculoid (TT) patients and MB skin smear negative Borderline-Tuberculoid (BT) patients, with or without leprosy reactions, with no sequel (defined by sensorial loss or motor deficit on hands or feet on Nerve Function Impairment (NFI) assessment), and the negative (–) group consisted of PB patients with or without leprosy reactions, but with sequel, together with all MB skin smear positive Borderline-Borderline (BB), Borderline-Lepromatous (BL) and Lepromatous-Lepromatous (LL) patients, regardless reactions or sequel. Additionally, in order to make other comparisons, patients were classified according to the bacilloscopic index (BI), a group with a low (<3) BI (LBI), and a group with high (≥3) BI (HBI) [28]. A sample of 110 healthy individuals from the same geographic area were included in the study as controls. All patients were informed about the study before signing informed consent forms. The project was approved by the Pará Federal University ethics committee (N° 197/07).

### DNA Extraction

DNA extraction was performed as previously described [29]. The DNA concentration was determined by spectrophotometry (Thermo Scientific NanoDrop 1000, NanoDrop Technologies, Wilmington, US).

### CYP2E1 Genotyping

Three *CYP2E1* polymorphisms, 1053C>T, 1293G>C and 7632T>A, were investigated using a TaqMan genotyping assay and analyzed by Real Time PCR 7500 (Life Technologies, CA, USA). The INDEL was investigated using conventional PCR methods, followed by visualization on an agarose gel. Specific PCR programs were established according to the annealing temperatures of the primers, and the amplifications were performed on a thermocycler Veriti 96 Well Thermal Cycler (Life Technologies, CA, USA). The alleles of the four *CYP2E1* polymorphisms investigated were defined using the official nomenclature, as described in <http://www.cypalleles.ki.se/cyp2e1.htm>.

### GSTM1 Genotyping

For amplification, a set of primers for *GSTM1F*/*GSTM1R* was investigated using conventional PCR methods (thermocycler Veriti 96 Well Thermal Cycler - Life Technologies, CA, US), followed by visualization on an agarose gel.

### Ancestry Informative Markers (AIM)

Individual interethnic admixture was estimated using a panel of 48 ancestry informative markers (AIMs), as previously described [30].

### Statistical Analyses

Estimations of linkage disequilibrium (D and D') and haplotypes and allelic frequencies were estimated with the M. Locus v. 2.0 software [31]. All other statistical analyses were performed using SPSS v. 12.0 (SPSS, Chicago, IL, USA), and results were considered statistically significant at p<0.05.

## Results

Demographic and clinical characteristics of the patients are shown in Table 1. Age, gender, sequel and clinical forms were all statistically significant when LBI and HBI were compared. Sequel

**Table 1.** Demographic and clinical characteristic of the sample according with Bacilloscopic Index.

Variables	Bacilloscopic Index <sup>c</sup> (N = 71)		p value (IC-95%)
	LBI n(%) = 32	HBI n(%) = 39	
Age <sup>a</sup>	35.2 ± 2.97	62.5 ± 3.42	<0.001
Gender <sup>b</sup> (M/F)	15(46.8%)/17(53.2%)	32(82%)/7(12%)	0.003
Sequel <sup>b</sup> (YES/NO)	12(37.5%)/20(62.5%)	30(76.9%)/9(23.1%)	0.001
Clinical Forms (PB/MB) <sup>b</sup>	10(31.2%)/22(68.8%)	0/39(100%)	0.002

<sup>a</sup>t-Test of Student;

<sup>b</sup>Fisher's Exact Test;

<sup>c</sup>Bacilloscopic Index (LBI = Bacilloscopic Index Low; HBI = Bacilloscopic Index High).

doi:10.1371/journal.pone.0047498.t001

occurred in 76.9% of the HBI patients whereas in the LBI group only 37.5% of the patients presented sequel.

There were 19 patients classified in the positive (+) group, of which nine were designated PB (two with reaction) and 10 were MB (four with reaction and all without sequel), while 52 patients comprised the negative (-) group, two of which were PB (all with reaction and sequel), and 50 were MB (35 with reaction and 40 with sequel) (Table 2). Concerning genotypic and allelic distribution of SNPs, a high (42.3%), statistically significant, frequency of the heterozygous genotype for the *CYP2E1\*6* allele was found among leprosy patients (Table 2). In the positive group, 63.2% (12 patients) exhibited this genotype, while in the negative group, a lower percentage (34.6%, 18 patients) was observed. The frequency of the wild-type *CYP2E1\*1A* and mutant *CYP2E1\*6* alleles in this population was 0.789 and 0.211, respectively, which

was statistically significant when the positive and negative groups were compared (Table 2).

Leprosy patients were also divided into two groups according to the bacilloscopic index (BI): a group with a low (<3) BI (LBI) and a group with high (≥3) BI (HBI). In addition to the analysis of the genotypic distribution of both *CYP2E1* and *GSTM1* markers in the LBI and HBI groups (Table 3), the combined effect of the two mutant alleles for the *CYP2E1* and *GSTM1* genes (*CYP2E1\*6/GSTM1\*0* and *CYP2E1\*5/GSTM1\*0*) was also analyzed. The mutant *CYP2E1\*5* allele was present in 37.5% of the patients in the LBI group, while the wild-type *CYP2E1\*1A* allele was observed in 92.3% of the patients in the HBI group. *GSTM1* gene analysis demonstrated that the mutant *GSTM1\*0* allele was present in 56.2% of the LBI group patients and in 38.5% of the HBI group patients, while the wild-type *GSTM1\*1* allele was present in 61.5% of the HBI group patients (Table 3). The analysis of the combined

**Table 2.** Genotypic and allelic distribution of SNPs on *CYP2E1* and *GSTM1* genes among patients grouped according to clinical evolution.

Genotype	Patients with Leprosy (%) (n = 71)	Group (+) (%) (n = 19)	Group (-) (%) (n = 52)	P* <sup>1</sup>	OR(95% IC)* <sup>2</sup>
<b><i>CYP2E1 (96 INDEL)</i></b>					
<i>*1C/*1C</i>	64 (90.1%)	16 (84.2%)	48 (92.3%)		1 (reference)
<i>*1C/*1D</i>	7 (8.9%)	3 (15.8%)	4 (7.7%)	0.375	0.444(0.09–2.202)
<i>CYP2E1*1C</i>	0.951	0.921	0.962		
<i>CYP2E1*1D</i>	0.049	0.079	0.038		
<b><i>CYP2E1 (7632)</i></b>					
<i>*1A/*1A</i>	41 (57.7%)	7 (36.8%)	34 (65.4%)		1 (reference)
<i>*1A/*6</i>	30 (42.3%)	12 (63.2%)	18 (34.6%)	<b>0.03</b>	0.309(0.103–0.922)
<i>CYP2E1*1A</i>	0.789	0.684	0.827		
<i>CYP2E1*6</i>	0.211	0.316	0.173		
<b><i>CYP2E1 (1053/1293)</i></b>					
<i>*1A/*1A</i>	56 (78.9%)	13 (68.5%)	43 (82.6%)		1 (reference)
<i>*1A/*5</i>	15 (21.1%)	6 (31.5%)	9 (17.4%)	0.206	0.453(0.135–1.513)
<i>CYP2E1*1A</i>	0.894	0.842	0.914		
<i>CYP2E1*5</i>	0.106	0.158	0.086		
<b><i>GSTM1</i></b>					
<i>GSTM1*1</i>	38 (53.5%)	12 (63.2%)	26 (50%)		1 (reference)
<i>GSTM1*0</i>	33 (46.5%)	7 (36.8%)	26 (50%)	0.423	1.714 (0.583–5.043)

\*<sup>1</sup>p-value;

\*<sup>2</sup>OR-odds ratio, CI-confidence interval.

doi:10.1371/journal.pone.0047498.t002

effect revealed that the *CYP2E1\*6/GSTM1\*0* genotypic combination was detected in 31.2% of the patients in the LBI group, while the *CYP2E1\*5/GSTM1\*0* genotypic combination was present in 28.1% of the patients in the LBI group; all were statistically significant when the different combinations were analyzed in the LBI or HBI groups.

Next, we performed a logistic regression analysis in which the two groups, LBI and HBI, were dependent variables and with covariables that could interfere with the results of PB and MB clinical forms. Although the results were not statistically significant for different variables, such as gender and sequel, they were significant when related to *CYP2E1\*1A/\*5* ( $p=0.0266$ ) and *GSTM1\*0* ( $p=0.0500$ ) genotypes. These results suggest a strong association between both mutations and LBI (Table 4).

To evaluate the presence of population substructure, we compared the clinical progression of leprosy patients (positive and negative groups, as well as high and low bacilloscopic index groups) with genomic ancestry, and the results showed no, significant. However, different frequencies were found for the investigated markers when leprosy patients were compared with a sample of healthy individuals from the same region (Table 5). The data showed that *CYP2E1\*5* allele is more frequent among the healthy individuals than among patients (0.196 and 0.106, respectively;  $X^2=6.85$ ;  $p=0.032$ ), while *CYP2E1\*6* allele is more common among patients than in the control sample (0.211 e 0.090, respectively;  $X^2=11.6$ ;  $p=0.003$ ).

### Discussion

Loss of sensation is the hallmark of leprosy diagnosis. It is well known that both, MB and PB patients may evolve to nerve

**Table 4.** Logistic regression analysis of the association between genetic markers and LBI/HBI response in leprosy patients.

Variable	$\beta$	S.E.	Wald	df	P	O.R (95%CI)
Age	0.0363	0.0209	3.0109	1	<b>0.0012</b>	1.0562 (0.9953–1.0804)
Gender	0.8705	0.7327	1.4113	1	0.2348	2.3881 (0.5680–10.407)
Sequel	0.8742	0.8405	1.0818	1	0.2983	2.3969 (0.4616–12.4468)
Clinical Form (PB/MB)	1.8598	1.1901	2.4421	1	0.1181	6.4226 (0.6233–66.1807)
<i>CYP2E1*1A/*5</i>	-1.6341	0.8457	3.7339	1	<b>0.0266</b>	0.1198 (0.0184–0.7816)
<i>CYP2E1*1A/*6</i>	1.0889	0.9079	1.4382	1	0.2304	2.9709 (0.5012–17.6087)
<i>GSTM1*0</i>	-1.3004	0.7025	3.4262	1	<b>0.05</b>	0.2724 (0.0687–1.0796)
African	-2.2083	1.8217	3.3266	1	0.4925	0.1099 (0.0002–60.2213)
European	0.9890	2.6275	0.1417	1	0.7066	2.6885 (0.0156–10.6548)
Amerindian	0.4674	2.5465	6.5769	1	0.8544	1.5958 (0.0108–11.0136)

$\beta$ , Coefficient Stimulation; **S.E.**, Standard Error; **df**, Degrees of Freedom; **p**, p-value; **OR**, Odds Ratio; **CI**, Confident Interval.  
doi:10.1371/journal.pone.0047498.t004

function impairment on the natural course of the disease [32]. It is usual - and comprehensible as an objective tool - to use BI to analyze the correlation between a specific gene or a genotypic combination and the evolution of leprosy. However, this cannot be the only parameter to evaluate in order to understand the disease behavior individually. HBI may indicate *M. leprae* ability to grow

**Table 3.** Combined and isolated genotypic distribution of *CYP2E1* gene (SNPs 1053T>C, 1293C>G and 7632T>A), and deletion (*GSTM1\*1/GSTM1\*0*) on gene *GSTM1* of patients classified accordingly to bacilloscopic index BI (LBI and HBI).

Genotype	Leprosy patients (n=71)	LBI (n=32)	HBI (n=39)	p*1	OR (95% IC)*2
<b><i>CYP2E1 (7632)</i></b>					
<i>*1A/*1A</i>	41 (57.74%)	16 (50%)	25 (64.1%)		1 (reference)
<i>*1A/*6</i>	30 (42.26%)	16 (50%)	14 (35.9%)	0.334	0.560 (0.216–1.452)
<i>CYP2E1*1A</i>	0.789	0.750	0.821		
<i>CYP2E1*5</i>	0.211	0.250	0.179		
<b><i>CYP2E1 (1053/1293)</i></b>					
<i>*1A/*1A</i>	56 (78.87%)	20 (62.5%)	36 (92.3%)		1 (reference)
<i>*1A/*5</i>	15 (21.13%)	12 (37.5%)	3 (7.7%)	<b>0.003</b>	0.139 (0.035–0.551)
<i>CYP2E1*1A</i>	0.894	0.813	0.962		
<i>CYP2E1*5</i>	0.106	0.187	0.038		
<b><i>GSTM1</i></b>					
<i>GSTM1*1</i>	38 (53.52%)	14 (43.8%)	24 (61.5%)		1 (reference)
<i>GSTM1*0</i>	33 (46.48%)	18 (56.2%)	15 (38.5%)	<b>0.0276</b>	0.486 (0.188–1.258)
<b><i>CYP2E1/GSTM1*3</i></b>					
<i>CYP2E1*1A/GSTM1*1</i>	58 (81.7%)	22 (68.8%)	36 (92.3%)		1 (reference)
<i>CYP2E1*6/GSTM1*0</i>	13 (18.3%)	10 (31.2%)	3 (7.7%)	<b>0.012</b>	0.183(0.045–0.740)
<i>CYP2E1*1A/GSTM1*1</i>	62 (87.3%)	23 (71.9%)	39 (100%)		1 (reference)
<i>CYP2E1*5/GSTM1*0</i>	9 (12.7%)	9 (28.1%)	-	<b>&lt;0.005</b>	0.371 (0.27–0.513)

\*1 p-value;  
\*2 OR-odds ratio, CI-confidence interval;  
\*3 Combined effect of mutant alleles of distinct genes.  
doi:10.1371/journal.pone.0047498.t003

**Table 5.** Allele and genotype distributions of CYP2E1 and GSTM1 genes within two samples from leprosy patients and healthy individuals.

Genotype	Patients with Leprosy (%) (n = 71)	Healthy Population (%) (n = 110)	$\chi^2$	p
<b>CYP2E1 (96 INDEL)</b>				
*1C/*1C	64 (90.1%)	96 (87.3%)		
*1C/*1D	7 (8.9%)	12 (10.9%)		
*1D/*1D	–	2 (1.8%)		
CYP2E1*1C	0.951	0.927	1.376	0.743
CYP2E1*1D	0.049	0.073		
<b>CYP2E1 (7632)</b>				
*1A/*1A	41 (57.7%)	90 (81.8%)		
*1A/*6	30 (42.3%)	20 (17.2%)		
*6/*6	–	–		
CYP2E1*1A	0.789	0.909	11.673	<b>0.003</b>
CYP2E1*6	0.211	0.091		
<b>CYP2E1 (1053/1293)</b>				
*1A/*1A	56 (78.9%)	70 (63.6%)		
*1A/*5	15 (21.1%)	37 (33.6%)		
*5/*5	–	3 (2.8%)		
CYP2E1*1A	0.894	0.805	6.855	<b>0.032</b>
CYP2E1*5	0.106	0.195		
<b>GSTM1</b>				
GSTM1*1	38 (53.5%)	53 (48.2%)		
GSTM1*0	33 (46.5%)	57 (51.8%)	1.136	0.722

doi:10.1371/journal.pone.0047498.t005

inside the host in order to keep transmission chain and strain survival, or may indicate the inability of the host in constrain bacterial growth.

Notwithstanding, the capacity of the human host immune system in dealing with leprosy infection with no sequel is rarely addressed. In the present study two groups of patients were examined, and a striking difference when BI or disease evolution were evaluated were observed in relation to the genes investigated herein. While *CYP2E1*<sup>7632</sup>\*1A/\*1A was associated to a worse disease progression, and the presence of the mutant *CYP2E1*<sup>7632</sup>\*1A/\*6 was associated with a good evolution, however, none of them were related to LBI or HBI. These findings suggest that different genes may be related to disease progression or bacterial growth inhibition mechanisms. Furthermore, *CYP2E1*<sup>1053/1293</sup>\*1A/\*1A was associated with HBI, while there no significant association was observed for clinical evolution analyses., *CYP2E1*<sup>1053/1293</sup>\*1A/\*5 was significantly associated with LBI and a better disease progression.

The availability of modern antibiotics can help us to better understand the disease, and it is reasonable to think that pharmacogenomics related genes may also be related to disease outcome in human hosts. One of these key drugs is rifampicin.

Rifampicin can be bacteriostatic at lower concentrations or bactericidal at higher concentrations. When used alone, mycobacterium can readily develop resistance to RIF, and therefore, treatment should not rely solely on this drug [15]. Its biotransformation occurs through a process of hepatic deacetylation, giving rise to the active metabolite desacetyl rifampicin [26]. RIF has a high capacity for inducing CYP450 isoforms, which contributes to a 40% reduction in half-life during the first half month of treatment and the acceleration of RIF deacetylation.

Therefore, this drug is capable of intensifying its own biotransformation, diminishing its plasmatic half-life when administered in multiple doses [33]. Studies of the *CYP2E1* gene indicate that *CYP2E1*\*6 and *CYP2E1*\*5 alleles are associated with a higher level of transcription and microsomal enzyme activity; therefore, they are implicated in enzymatic biotransformation activity augmentation [34–36], consequently decreasing the half-life of RIF.

Our results show that among the patients grouped according to clinical progression, the heterozygous genotype *CYP2E1*\*1A/\*6 was present in 63.2% of the individuals in the (+) group. The OR analysis of the *CYP2E1*\*6 allele demonstrated that this polymorphism provides protection to those individuals in the (+) group (Table 2).

We hypothesize that the *CYP2E1*\*6 allele could increased the rate of rifampicin metabolism. Augments the biotransformation by CYP450 enzymes and raising the levels of the active metabolite desacetyl rifampicin, which has a higher bactericidal activity. Therefore, individuals with this mutation could more efficiently combat *M. leprae*.

A significant difference was found between healthy individuals and patients for the *CYP2E1*\*6 allele, which is more common among leprosy patients ( $X^2 = 11.6$ ;  $p = 0.003$ ). Since this association was unknown, more studies are necessary to confirm these results (Table 5).

Among leprosy patients, *CYP2E1*\*5 allele was more frequent in the LBI group. This allele was also more frequent in healthy subjects when compared to leprosy individuals. These results taken together suggest that *CYP2E1*\*5 is a protection factor that might be involved with bacterial growth inhibition (Table 5).

For the *GSTM1* gene, the null genotype *GSTM1*\*0 was present in 56.2% of the LBI group. The compound distribution of the two

mutant alleles *CYP2E1\*5/GSTM1\*0* was present in 28.1% of LBI the patients, while *CYP2E1\*6/GSTM1\*0* was present in 31.2%. The estimated OR suggest that mutant alleles confer protection for LBI individuals.

Taken together, our results suggest that the *CYP2E1\*5*, *CYP2E1\*6* and *GSTM1\*0* alleles may be considered as susceptibility markers for leprosy, and their distribution should be further investigated, as their presence seems to confer protection from *M. leprae*.

## References

- Alcais A, Mira M, Casanova JI, Schurr E, Abel I (2005) Genetic dissection of immunity in leprosy. *Current Opinion in Immunology* 17: 44–48.
- Moreira MBR, Pena GO, Pereira GFM, Madalena M (2002). Ministério da Saúde, Secretaria de Políticas de Saúde, Guia para o controle da hanseníase. ISBN 85-334-0346-1.
- Stretch R (1999) Presentation and treatment of Hansen's disease. *Nurs Times* 95(29): 46–7.
- Moreira AS, Ribeiro dos Santos RC, Bastos RR, Silva JV, Santos PM (2006) Conjunctival bacilloscopy in leprosy diagnosis and follow-up. *Arq Bras Oftalmol* 69(6): 865–9.
- Goulart IMB, Arbex GL, Carneiro MH, Rodrigues MS, Gadia R (2002) Adverse effects of multidrug therapy in leprosy patients: a five-year survey at a Health. *Rev Soc Bras Med Trop* 35(5): 453–60.
- Ridley DS, Jopling WH (1966) Classification of leprosy according to immunity: a five-group system. *International Journal of Leprosy Other Mycobact. Disease* 34: 255–273.
- Kaur MR, Lewis HM (2006) Hidradenitis suppurativa treated with dapson: a case series of five patients. *J Dermatol Treat* 17: 211–213.
- Morrison NE, Morley GM (1976) The mode of action of clofazimine: DNA binding studies. *Int. J. Lepr* 44: 133–135.
- Yano T, Bratinova SK, Teh JS, Winkler J, Sullivan K, Isaacs A, et al. (2011) Reduction of Clofazimine by Mycobacterial Type 2 NADH:Quinone Oxidoreductase. *Jour. Bio. Chem.* 286: 10276–10287.
- Jopling WH (1983) Side effects of antileprosy drugs in common use. *Leprosy Review* 54: 261–270.
- Jopling WH (1985) References to "side-effects of antileprosy drugs in common use". *Leprosy Review* 56: 61–70.
- Pfaltzgraff RE, Bryceson A (1985) Clinical Leprosy. In: Hastings RC. *Leprosy*. New York p.134–136.
- Opromolla DVA (1997) Terapêutica da hanseníase. *Medicina Ribeirão Preto* 30: 345–350.
- Souza CS (1997) Hanseníase: formas clínicas e diagnóstico diferencial. *Medicina Ribeirão Preto* 30: 325–334.
- Rivers EC, Mancera RL (2008) New Anti-Tuberculosis Drugs with Novel Mechanisms of Action. *Curr Med Chem*; 15(19): 1956–67.
- Rivera RC, López CR, Herranz AB, Cevallos MA (2010) Analysis of the Mechanism of Action of the Antisense RNA That Controls the Replication of the repABC Plasmid p42d. *Journal of Bacteriology* 192: 3268–3278.
- Hasler JA, Estabrook R, Murray M, Pikuleva I, Waterman M, Capdevila J, et al. (1999) Human cytochromes P450. *Molecular Aspects of Medicine* 20: 1–137.
- Yamada S, Tang M, Richardson K, Halaschek-Wiener J, Chan M, et al. (2009) Genetic variations of NAT2 and CYP2E1 and isoniazid hepatotoxicity in a diverse population. *Pharmacogenomics* 10(9): 1433–45.
- Kayaaltı Z, Söylemezoglu T (2010) Distribution of ADH1B, ALDH2, CYP2E1\*6, and CYP2E1\*7B genotypes in Turkish population. *Alcohol* 1: 9–12.
- Uematsu F, Ikawa S, Kikuchi H, Sagami I, Kanamaru R, et al. (1994) Restriction fragment length polymorphism of the human CYP2E1 (cytochrome P450IIE1) gene and susceptibility to lung cancer: possible relevance to low smoking exposure. *Pharmacogenetics* 4(2): 58–63.
- Widersten M, Pearson WR, Engstrom A, Mannervik B (1991) Heterologous expression of the allelic variant Mu-class glutathione transferases m and y. *Biochemical Journal* 276: 519–524.
- Moyer AM, Salavaggione OE, Hebbing SJ (2007) Glutathione S-Transferase T1 and M1: Gene Sequence Variation and Functional Genomics. *Clin Cancer Res* 13: 7207–7216.
- Li L, Yang L, Zhang Y, Xu Z, Qin T, et al. (2011) Detoxification and DNA repair genes polymorphisms and susceptibility of primary myelodysplastic syndromes in Chinese population. *Leuk Res* 35(6): 762–5.
- Cho HR, Uhm YK, Kim HJ, Ban JY, Chung JH, et al. (2011) Glutathione S-transferase M1 (GSTM1) polymorphism is associated with atopic dermatitis susceptibility in a Korean population. *Int J Immunogenet* 38(2): 145–50.
- Konwar R, Manchanda PK, Chaudhary P, Nayak VL, Singh V, et al. (2010) Glutathione S-transferase (GST) gene variants and risk of benign prostatic hyperplasia: a report in a North Indian population. *Asian Pac J Cancer Prev* 11(4): 1067–72.
- Hardman JG, Limbird LE (2004) Goodman & Gilman as bases farmacológicas da terapêutica. Raven Press: Rio de Janeiro. 80 p.
- Etienne D, Pieter F, Cairns S (2000) Report of the International Leprosy Association Technical Forum: Prevention of disabilities and rehabilitation. *Lepr Rev* 73: S35–S43.
- Vaishali BN, Usha BN, Swati M, Rao P (2011) Evaluation of significance of skin smears in leprosy for diagnosis follow-up, assessment of treatment outcome and relapse. *Asiatic Jour. of Biotech. Resources* 2(5): 547–552.
- Sambrook J, Fritsch F, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spr Harb Laboratory, NY. 2nd edition.
- Santos NPC, Ribeiro-Rodrigues EM, Ribeiro-dos-Santos AKC (2010) Assessing individual interethnic admixture and population substructure using a 48 insertion-deletion ancestry informative markers panel. *Hum Mutat.* 31(2): 184–90.
- Long J (1999) Multiple Locus Haplotype Analysis, version 2.0. Software and documentation distributed by the author. Section on Population Genetics and Linkage, Laboratory of Neurogenetics, NIAAA, National Institutes of Health, Bethesda.
- Crawford CL (2010) Historical aspects of leprosy. *Clin Infect Dis* 51: 476–7.
- Douglas JG, Macleod MJ (1999) Pharmacokinetic factors in the modern drug treatment of tuberculosis. *Clin Pharmacokinet* 37: 127–46.
- Tsutsumi M, Takada A, Wang JS (1994) Genetic polymorphisms of cytochrome P4502E1 related to the development of alcoholic liver disease. *Gastroenterology* 107: 1430–1435.
- Yang BM, O'Reilly DA, Demaine AG, Kingsnorth AN (2001) Study of polymorphisms in the CYP2E1 gene in patients with alcoholic pancreatitis. *Alcohol* 23: 91–97.
- Wang SM, Zhu AP, Li D, Wang Z, Zhang P, et al. (2009) Frequencies of genotypes and alleles of the functional SNPs in CYP2C19 and CYP2E1 in mainland Chinese Kazakh, Uygur and Han populations. *J. Hum. Genet* 54: 372–375.

## Acknowledgments

Special thanks the donors of samples (leprosy patients from northern Brazil), who enable this study to be carried out, CAPES (Coordenação de Aperfeiçoamento Pessoal de Nível Superior) and UFPA (Universidade Federal do Pará).

## Author Contributions

Conceived and designed the experiments: CS SS MH ARS. Performed the experiments: PP CS DOA ARS. Analyzed the data: PP NS SS. Contributed reagents/materials/analysis tools: CS SS ARS. Wrote the paper: PP CS ARS.