Is nullity for Glutathione S-transferase genes GSTT1 and GSTM1 protective against leprosy?

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Summary

Introduction: Leprosy is a chronic and progressive infectious disease caused by Mycobacterium leprae. The generation of free radicals called reactive oxygen species (ROS), which promote destruction of the bacilli within macrophages, is an effective defence mechanism developed by the host. In parallel, the glutathione S-transferase (GST) multifamily of enzymes constitutes an important antioxidant system for ROS detoxification and protection against toxicity. Therefore, GST null genotype individuals could reduce the detoxification of ROS, increasing host effectiveness for M. leprae destruction.

Objectives: To evaluate polymorphisms in the GSTT1 and GSTM1 genes as human leprosy susceptibility modulators.
Introduction

Leprosy is a slow and progressive infectious disease caused by *Mycobacterium leprae*, an obligate intracellular bacillus, with a broad clinical and immunological spectrum of disease. The tuberculoid form (TT) is characterised by isolated skin lesions with or without occasional intracellular bacilli (pangenic bacillary form) and a lymphocytic T helper 1 (Th1) cellular immune response. On the other hand, lepromatous leprosy (LL) is characterised by the presence of profuse bacilli (multibacillary form) in the skin and a lymphocytic T helper 2 (Th2) humoral immune response. In addition, four intermediate forms, borderline tuberculoid (BT), midborderline (BB), borderline lepromatous (BL) and indeterminate (I), are observed.²

The major defence against infection by any microorganism is the macrophage system. The generation of free radicals produced by phagocytes, called reactive oxygen species (ROS), which promote the destruction of *M. leprae*, is an effective defence mechanism developed by the host.² In parallel, cells have a comprehensive system of antioxidant defences to prevent free radical formation, and the glutathione S-transferase (GST) multifamily of enzymes constitutes an important antioxidant system for ROS detoxification and protection against toxicity. Therefore, GST null genotypes could reduce the detoxification of ROS, increasing its effectiveness for microorganism destruction.⁴

Glutathione S-transferases (GSTs) constitute a multifunctional family of enzymes that are coded by at least eight distinct loci: α (GSTA), μ (GSTM), θ (GSTT), π (GSTP), υ (GSTV), κ (GSTK), ω (GSTO), and ζ (GSTZ); GSTT1 and GSTM1 are the most studied.⁵ The GSTT1 gene is located on chromosome 22 (22p 11.2), while the GSTM1 gene is located on chromosome 1 (1p 13-3). The GSTT1 and GSTM1 enzyme activity deficiency results from the inherited absence of the GSTT1 or GSTM1 genes (GSTT1 or GSTM1 null genotypes).⁶,⁷

In this context, several authors sought to investigate the possible correlation between human cancer susceptibility and GST gene polymorphisms, including bladder, kidney, liver, gastric, tobacco-related (lung and oral cavity), colorectal, brain and skin tumours, among others, with contradictory results.⁸ Some of these disparities seem to be related to differential xenobiotic exposure or even to the frequency of the GSTM1 and GSTT1 polymorphisms, since both can be influenced by population ethnicity.⁹
The purpose of the present study was to evaluate whether GSTT1 and GSTM1 genes polymorphisms (presence or absence) can influence human susceptibility to leprosy and/or modulate the severity of this disease in a Southeast Brazilian population.

Materials and methods

Subjects

The study population was composed of subjects older than 18 years, belonging to any ethnic group. They were invited to participate after signing an informed consent form, approved by the Ethical Research Board from the Faculdade de Medicina de São José do Rio Preto – FAMERP (number 3898/2006).

Patients

This study represents a case-control investigation of 218 leprosy patients (n = 218). 103 female and 115 male, recruited from two leprosy treatment reference centres in São José do Rio Preto city, located in the Northwestern region of São Paulo state, Southeast Brazil: the Núcleo de Gestão Assistencial 60 (NGA-60) and the Sanitary Dermatological Service of the Hospital de Base Outpatients Clinic (HB-DAS), FUNFARME Foundation. The Hospital de Base (HB) is a tertiary care hospital catering to a large population from the Northwestern region of São Paulo State, Brazil, representing, along with the NGA 60, the regional reference centre for leprosy diagnosis and treatment. Together, these two centres concentrate leprosy health care in the São Paulo State Health Regional Department XV (DRS XV), which includes 101 cities. All patients were invited to participate among those who started leprosy treatment in the municipality from November 2006 until April 2010. Leprosy diagnosis was based on clinical assessment as evaluated by physicians, and detection of acid-fast bacilli either in skin-slit smears or in skin biopsies. Patients were classified according to the Ridley-Jopling Scale† and also as paucibacillary (PB) or multibacillary (MB), with subsequent treatment according to World Health Organization (WHO) specifications. The PB group (BT, TT and T) included 96 cases, while the MB group (LL, BL and BB) included 122 cases. General characteristics of the patient groups are summarised in Table 1.

<table>
<thead>
<tr>
<th>Classification used for treatment</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paucibacillary</td>
<td>96 (44.8)</td>
</tr>
<tr>
<td>Multibacillary</td>
<td>122 (56.4)</td>
</tr>
<tr>
<td>Clinical forms (Ridley and Jopling)</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>50 (41.1)</td>
</tr>
<tr>
<td>BL</td>
<td>13 (6.0)</td>
</tr>
<tr>
<td>BB</td>
<td>19 (8.7)</td>
</tr>
<tr>
<td>BT</td>
<td>19 (8.7)</td>
</tr>
<tr>
<td>TT</td>
<td>63 (28.6)</td>
</tr>
<tr>
<td>T</td>
<td>14 (6.4)</td>
</tr>
</tbody>
</table>

LL, lepromatous leprosy; BL, borderline lepromatous; BB, borderline; BT, tuberculoid leprosy; TT, tuberculoid leprosy; T, indeterminate.
CONTROL GROUP

Two hundred and forty four (n = 244) unrelated subjects, 110 female and 134 male, from the same endemic area as the patients, were selected among blood bank donors from the HB Blood Bank, in the same municipality, as controls. They were matched with leprosy patients by age (± 5 years) and gender. None of them was under continuous medication.

DNA EXTRACTION AND GSTTI AND GSTM1 GENOTYPING

The genomic DNA was obtained from peripheral blood according to Miller et al.\textsuperscript{11} with modifications. The genotyping of the GSTTI and GSTM1 polymorphisms was performed by using a multiplex polymerase chain reaction (PCR) with co-amplification of the CYP1A1 gene as an internal control for successful amplification.\textsuperscript{2} Briefly, PCR amplifications were carried out in 50 \( \mu \)l reactions using 25–100 ng of genomic DNA as template in tubes containing 1 U Taq DNA polymerase with 1X of the buffer as supplied by the manufacturer (Invitrogen, CA, USA), 1.5 mM of MgCl\(_2\), 0.2 mM of each dNTP and 0.5 \( \mu \)M of each primer. Amplification cycles included a denaturing step at 94°C for 5 minutes followed by 35 cycles set at 94°C for 2 minutes, 62°C for 1 minute and 72°C for 1 minute and a final extension at 72°C for 5 minutes. The PCR products were electrophoresed in 2% agarose gel and visualized by ethidium bromide staining. DNA from samples positive for GSTM1 and GSTTI genotypes yielded bands of 215 bp and 480 bp, respectively, while the internal positive control (CYP1A1) PCR product corresponded to 315 bp.

The associations of the GSTTI and GSTM1 polymorphisms in patients and control subjects were carried out by weighted logistic regression models. Results are shown as odds ratios (OR) with 95% confidence intervals (95% CI). The significance of the allele frequency difference between the MB and PB groups and GSTTI, GSTM1 genotypes was analysed using Pearson’s Chi-square test. \( P \)-values < 0.05 were considered statistically significant.

Results

The mean age of patients and controls were 55 years ± 14.2 and 51.4 years ± 16.6 (mean ± standard deviation), respectively. There were no statistically significant differences between cases and controls, indicating a well-matched population.

After comparing the genotypes obtained for both study groups, we could detect that the presence of both genes (GSTM1 and GSTTI positive genotype) enhanced the risk for leprosy (OR 1.824, 95% CI 1.197–2.779) whereas the frequency of the GSTTI/GSTM1 null genotypes was significantly higher among control subjects than among patients (\( P = 0.0223 \)). The GSTTI positive genotype frequency was significantly increased in leprosy patients when compared with control subjects (\( P = 0.0097 \)), a phenomenon that was not observed when the GSTM1 positive genotype frequency was considered (\( P = 0.5492 \)). The distribution of GSTTI and GSTM1 alleles in patients and control subjects is shown in Table 2.

The same results were obtained when the variables gender and age were included in the logistic regression model. In addition, no differences were observed in the frequency of the GSTTI/GSTM1 genotypes between MB and PB groups (\( P > 0.05 \)).
Table 2. Genotypic frequencies among patients versus controls and MB versus PB forms of leprosy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n = 218)</th>
<th>Controls (n = 244)</th>
<th>P-value</th>
<th>MB (n = 52)</th>
<th>PB (n = 56)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTT1/GSTM1 null</td>
<td>0.430</td>
<td>0.188</td>
<td>0.0223*</td>
<td>0.089</td>
<td>0.634</td>
<td>0.7958</td>
</tr>
<tr>
<td>GSTT1</td>
<td>0.388</td>
<td>0.672</td>
<td>0.0067*</td>
<td>0.795</td>
<td>0.781</td>
<td>0.9352</td>
</tr>
<tr>
<td>GSTM1</td>
<td>0.495</td>
<td>0.463</td>
<td>0.548</td>
<td>0.580</td>
<td>0.489</td>
<td>0.9870</td>
</tr>
</tbody>
</table>

n = number of individuals, *P* = significance level *P* < 0.05. Comparisons between cases and controls, multibacillary (MB) and paucibacillary (PB) were performed using Chi-square test. *P* < 0.05 is significant.

Discussion

Several studies in different populations around the world have investigated leprosy susceptibility and disease severity through human genetic susceptibility. The majority aimed at determining the role of proteins and enzymes involved in Th 1 and Th 2 immune responses, vitamin D receptor gene variations, Parkin (PARK2) and parkin co-regulated genes (PACRG), and also the Toll-like receptor gene polymorphisms, with conflicting results. As far as we know, our study is the first to investigate the GST gene polymorphisms as possible modulators of susceptibility and/or severity in leprosy. Therefore, it is not an easy task to discuss the results here obtained, which will be explained based on the GST enzymes function versus the well-recognised mechanism of *M. leprae* elimination - the macrophage defense through ROS.

In the present investigation, we analyzed the combined genotypes of GSTM1 and GSTT1 genes to evaluate the possibility of a contribution to the severity of leprosy or to a modulation of the human susceptibility to this disease. In fact, the combination of the GSTT1 and GSTM1 null genotypes was more frequent in the control group than in the leprosy group. We also suggest an association of GSTT1 positive genotype and susceptibility to developing leprosy.

As a matter of fact, GSTs are a class of abundant proteins that promote cellular defence against different artificial and naturally occurring environmental agents by catalysing the conjugation of glutathione to various electrophiles and xenobiotics, including ROS. Recently, Kawamura et al targeting cancer treatment, by a combination of proteomic profiling and affinity purification, together with subsequent biochemical assays, described that the mechanism by which a small molecule induces ROS is the depletion of cellular glutathione. Together, our data led us to the proposition that the absence of GSTs, with a consequent maintenance of high levels of intracellular ROS, can contribute to *M. leprae* elimination and, therefore, reduce leprosy susceptibility. On the other hand, we could not establish any statistically significant association between these polymorphisms and clinical subtypes of leprosy. Indeed, if GST nullity plays an important role in bacilli destruction, as suggested, this could be more likely a protective effect, which would act before the establishment of a multi or paucibacillary forms of the disease.

GST gene polymorphisms were extensively studied in case-control studies which investigated human susceptibility to several types of cancer in different populations with both positive and negative associations, mainly attributed to particularities concerning
environmental exposure to carcinogens and populations with diverse ethnic composition. While the first do not seem to represent an important issue in the present study, the bias generated in case-control studies based on polymorphisms investigated in human admixed populations can be of major concern. The lack of individual genotyping of ethnicity markers before case-control matching was a limitation of this study that we acknowledge.

Further studies with a large cohort and a careful case-control match according to ancestry informative markers to control ethnicity will shed additional light on the role of GSTM1 and GSTT1 in leprosy.

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References


