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Research paper

The *GATA3* gene is involved in leprosy susceptibility in Brazilian patients

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ABSTRACT

Leprosy outcome is a complex trait and the host–pathogen–environment interaction defines the emergence of the disease. Host genetic risk factors have been successfully associated to leprosy. The 10p13 chromosomal region was linked to leprosy in familial studies and *GATA3* gene is a strong candidate to be part of this association. Here, we tested tag single nucleotide polymorphisms at *GATA3* in two case–control samples from Brazil comprising a total of 1633 individuals using stepwise strategy. The A allele of rs10905284 marker was associated with leprosy resistance. Then, a functional analysis was conducted and showed that individuals carrying AA genotype express higher levels of GATA-3 protein in lymphocytes. So, we confirmed that the rs10905284 is a locus associated to leprosy and influences the levels of this transcription factor in the Brazilian population.

1. Introduction

Leprosy is still a public health problem exhibiting around 225,000 new cases by year, while Brazil ranks second in the number of patients in the world (World Health Organization, 2014).

The disease is caused by the intracellular pathogen *Mycobacterium leprae*, which has tropism for macrophages in skin and Schwann cells in the nerves turning leprosy the primary cause of nerve incapacity due to an infectious agent. *M. leprae* exhibits an extremely conserved and compact genome associated with long generation time. This bacillus is well adapted to the humans, but a large range of outcomes may emerge from the interplay between host and mycobacterium, and individuals can either eliminate the bacteria or develop infection.

The spectrum of clinical forms of leprosy is based on clinical, immunological, microbiological and histopathological criteria. The patients can evolve to a localized form, known as tuberculoid (TT), or to a disseminated form, named lepromatous (LL). This polar pattern of leprosy, TT or LL disease, resembles a Th1 or Th2 profiles of immune response, respectively (Modlin, 1994). The TT patients prevent the replication of the bacillus by a cell mediated immune (CMI) response, while LL patients present a poor CMI resulting in bacterial persistence, replication and spread throughout the body. Focusing on the treatment, the World Health Organization (WHO) proposed a clinical classification based on the number of skin lesions, that includes paucibacillary (PB) and multibacillary (MB) forms (World Health Organization, 1998).

Due to the low genetic variability of the pathogen and the diversity of phenotypes emerging from its interaction to the host, it is assumed that the host genetic background has a main role in the control of the leprosy development (Monot et al., 2005; Alter et al., 2011). In fact, twin studies showed the genetic influence in leprosy, revealing the higher concordance rate of disease per se and clinical forms in monozygotic than dizygotic pairs (Chakravartti and Vogel, 1973). Afterwards, complex segregation analysis demonstrated the presence of a major gene controlling the disease susceptibility (Abel and Demenais, 1988; Lázaro et al., 2010). Noteworthy, genetic epidemiology approaches are consistently emphasizing the presence of a genetic control in leprosy. A variety of genes and single nucleotide polymorphisms (SNPs) influencing the susceptibility to leprosy were identified in different populations: *TNF/LTA/HLA* (Roy et al., 1997; Santos et al., 2002; Cardoso et al., 2011; Vanderborght et al., 2007; Alcaïs et al., 2007), *IL10* (Santos et al., 2002; Malhotra et al., 2005; Pereira et al., 2009), *PARK2* and *PACRG* (Mira et al., 2004), *NOD2* (Zhang et al., 2009; Grant et al., 2012; Sales-Marques et al., 2014), *CCDC122* (Zhang et al., 2009; Grant et al., 2012; Sales-Marques et al., 2014), *IFNG* (Cardoso et al., 2010), and *TLR1* (Marques et al., 2013).

Two genome-wide linkage studies for leprosy pointed peaks at the 10p13 chromosomal region. The first one, from India, described the peak linked to the leprosy per se (Siddiqui et al., 2001). However, the second, from Vietnam, concluded that this region is involved in PB leprosy (Mira et al., 2003). The region contains many candidate genes and among them some were associated to leprosy. Markers at the *MRC1*

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(mannose receptor, C type 1) gene were associated to the disease per se or clinical forms in Vietnamese, Brazilian and Chinese populations (Alter et al., 2010; Wang et al., 2012). Recently, a high-density association scan was performed in two family-based samples from Vietnam, and two SNPs were associated to MB leprosy at the *CUBN* and *NEBL* genes (Grant et al., 2014).

GATA3 gene is located at 10p15 chromosomal region and is a strong candidate gene to leprosy susceptibility since encodes the GATA-3 transcription factor that induces Th2 immune response that favors the permissiveness to *M. leprae* replication and spread (Yang et al., 2014). Besides, GATA-3 acts in other points of innate and adaptive immunity such as T cells development, innate lymphoid cell development and function, regulatory and CD8 + T cells as well as thymic natural killer cells (Tindemans et al., 2014).

Here, we investigated the association of *GATA3* gene to leprosy using a stepwise strategy in two case–control samples from Brazil enrolling 922 patients and 737 controls. Then, we verified the functional effect of rs10905284 SNP at *GATA3* on the expression of this gene.

2. Methods

2.1. Subjects and study design

The genetic epidemiology study enrolled 1659 individuals distributed in two independent case–control samples. A stepwise strategy was adopted using a discovery and a replication population. These populations are well-characterized in previous studies (Marques et al., 2013; Sales-Marques et al., 2014) and are detailed at the Supplementary Table 1.

First, we tested seven markers in 768 individuals in the discovery sample from endemic region of Rondonopolis, Mato Grosso State, located at the west center of Brazil. Four-hundred eleven cases were assembled from a specialized clinic and three-hundred fifty seven healthy controls were recruited during the campaigns for detecting new leprosy cases in public places of the region.

Then, SNPs associated in the discovery sample were investigated in a replication sample comprising 871 individuals from São Paulo State, located at the southeastern region of Brazil that presents lower number of leprosy cases. All leprosy cases (511 patients) were enrolled from the outpatient clinic specialized in leprosy from Instituto Lauro de Souza Lima. The control group (380 individuals) was derived from blood donors.

Patients were classified according to the Ridley and Jopling criteria (Ridley and Jopling, 1966). However, to evaluate the genetic effect of *GATA3* SNPs in the severity of leprosy, patients were classified into PB and MB according to bacillary index. Patients presenting a positive index were classified as MB and a negative index was condition to the PB patients. The leprosy subtype distribution was 75% MB and 23% PB in the discovery sample, and 76% MB and 19% PB in the replication sample.

The maximum power of our sample to reveal the same effect of the rs10905284 observed to leprosy per se in the leprosy subsets was 0.83, using an additive model and one-side type I error rate of 0.05 for PB and MB subsets (Table 1). In practice, a power of 80% to detect what we want to test is advisable (Pacheco and Moraes, 2009).

Table 1
Power to detect the association of OR = 0.73 in the PB (n = 185) and MB subsets (n = 655) considering the minor allele frequencies of the combined sample.

Stratum	MAF[a]	Model[b]	Power ($\alpha = 0.05$)	Power ($\alpha = 0.01$)
PB	0.44	ADD	0.83	0.61
MB	0.43	ADD	0.83	0.61

Abbreviation: PB — paucibacillary, MB — multibacillary.
[a] MAF — minor allele frequency of each stratum in the combined sample.
[b] Genetic model — ADD (additive).

The study of the functional effect of rs10905284 at *GATA3* gene was based on the homozygous genotypes. We studied healthcare workers of the Instituto Lauro de Souza Lima, all of them were women (eight presenting the AA genotype and eight presenting the CC genotype).

This work was approved by the Ethics Committee from Instituto Lauro de Souza Lima and all the protocols required were followed.

2.2. SNPs selection

To select tag SNPs to cover *GATA3* gene we used the tagger multimaker method considering the minor allele frequency of 0.1 in the Yoruba population and a cutoff r^2 of 0.8 in International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/). Thus, seven tag SNPs across the *GATA3* gene were picked, as follows: rs10905284, rs1399180, rs2280015, rs3781094, rs3802604, rs444929 and rs569421.

2.3. DNA extraction and SNP genotyping

Genomic DNA was extracted from peripheral blood leukocytes samples by salting-out method. The genotyping of the discovery sample was made using the GoldenGate assay with VeraCode technology in BeadXpress reader (Illumina, Inc., San Diego, CA, USA). For the replication sample and to select the genotypes for the functional study, the genotyping was made by allelic discrimination using fluorogenic probes in TaqMan SNP genotyping assay in StepOnePlus equipment (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

2.4. Peripheral blood mononuclear cell (PBMC) culture

Twenty-four milliliters of peripheral blood were collected from each individual in heparinized tubes by venipuncture. PBMCs were obtained by gradient centrifugation using Histopaque - 1077 (Sigma-Aldrich – Saint Louis – USA) and total cell number was estimated by counting in a Neubauer chamber. The concentration of the cell suspension was adjusted to 1.2×10^6 cells/ml and the cells were cultured for 48 h in 24-well polystyrene plates at 37 °C in a humidified atmosphere of 5% CO_2. RPMI 1640 medium supplemented with 10% serum fetal bovine and 1% antibiotics was used. Phytohemagglutinin (PHA, 8 μg/ml) or *M. leprae* sonicated antigen (ML, 10 μg/ml) were used as stimuli; unstimulated cultures (US) were kept on the same conditions as controls. After 48 h, the supernatants were collected and stored at − 80 °C for cytokine assay. Cells were used to analyze GATA-3 expression by flow cytometry.

2.5. Flow cytometry

For the analysis of GATA-3 expression, cells were harvested, washed in phosphate buffered saline (PBS) pH 7.4 and submitted to blockage of nonspecific sites for 1 h at 4 °C with normal mouse serum and normal human serum. Lymphocytes were labeled employing anti-CD4 FITC and CD69 PE (BD Biosciences, San Jose, CA, USA) for 1 h at 4 °C in the dark. The cell suspension was washed twice with PBS containing 5% fetal bovine serum. For GATA-3 labeling, cells were fixed and permeabilized using a specific kit (Buffer TranscriptionFactor Set BD Biosciences, San Jose, CA, USA) and then incubated with anti-GATA-3 antibody labeled with AlexaFluor-647 (BD Biosciences, San Jose, CA, USA) for 1 h at 4 °C in the dark. To control the specificity of the reactions we employed unrelated antibodies presenting the same isotype and conjugated with the same fluorochrome (isotype controls, BD Biosciences, San Jose, CA, USA).

Data collection was performed in a FACSCalibur flow cytometry (BD Biosciences, San Jose, CA, USA), including a minimum of 10,000 events. For data analyses we used the FlowJo software version 7.6.5 (2011) and consider the mean fluorescence intensity values (MFI).