Association of the rs4646994 in ACE gene with susceptibility to tuberculosis in a region of the Brazilian Amazon

Débora Chistina Ricardo Fernandes Porchera

Universidade Federal do Pará

Ana Cristina Oliveira Braga

Universidade Federal do Pará

Diana Feio Veiga Borges Leal

Universidade Federal do Pará

Pablo Diego Carmo Pinto

Universidade Federal do Pará

Mayara Natália Santana da Silva

Universidade Federal do Pará

Lucas Cauê Bezerra Santos

Universidade Federal do Pará

Cintia Helena Braga da Silva

Universidade Federal do Pará

Giovana Escribano Costa

Universidade Federal do Pará

Maria Clara Costa Barros

Universidade Federal do Pará

Cleonardo Augusto Silva

Universidade Federal do Pará

Paulo Pimentel Assumpção

Universidade Federal do Pará

Ândrea Ribeiro Santos

Universidade Federal do Pará

Sidney Emanuel Batista dos Santos

Universidade Federal do Pará

Marianne Rodrigues Fernandes

Universidade Federal do Pará

Ney Pereira Carneiro dos Santos (✉ npcsantos.ufpa@gmail.com)

Universidade Federal do Pará

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Abstract

Background: Tuberculosis is a global public health issue. Recent studies suggested association of host genetic factors to tuberculosis susceptibility in many populations. Polymorphisms can influence the host immune responses to tuberculosis. This study was designed to investigate the association of seventeen genetic polymorphisms with susceptibility and severity to tuberculosis in a sample of Brazilian population.

Methods: This case-control study encompassed 283 active tuberculosis patients and 145 healthy subjects that had contact with the bacillus. Genotyping of 13 INDELs polymorphisms and 4 SNPs was achieved using Multiplex PCR method and TaqMan SNP Genotyping Assays.

Results: Of the 17 investigated markers, only the *ACE* marker (rs4646994) showed significant differences between cases and controls.

Conclusions: The DEL/DEL (deletion /deletion) genotype of the *ACE* marker configured a protection factor for the development of tuberculosis. As there is no data in the recent literature relating this polymorphism with tuberculosis in a Brazilian population, our study has become unprecedented.

Background

Tuberculosis is a public health problem despite the existence of international control programs. About 10 million cases occurred worldwide and eventually 2-3 million die of the disease every year according to World Health Organization (WHO). Nearly one-quarter of the world population is estimated to be infected with *Mycobacterium tuberculosis* (Mtb) in 2020, the pathogen responsible for TB disease in humans, but only 5-15% of these people will fall ill with active TB disease [1–3].

The *M. tuberculosis* is not the only determining factor toward disease progression. Currently, there is an increasing focus in genomic research of human. Genetic factors play a major role in the pathogenesis and polymorphisms in the different genes can influence and modulate the immune responses [4]. Genetic polymorphisms, such as single nucleotide polymorphisms (SNPs) and insertion/deletion (INDELs) can affect gene transcription, mRNA processing, levels of cytokine production and gene expression [5, 6].

INDELs variation at protein-coding genes and in other functionally constrained regions of the genome is likely to form the main genetic background to phenotypic variation, possibly causing diseases like cancer, tuberculosis and Alzheimer's disease [7–9]. Many studies have suggested that polymorphisms in immune response genes such as interleukins [10–12] and *CCR5* [13, 14], apoptotic pathways such as *TP53* [15] and metabolism such as *UGT1A1* are associated with TB among different populations [16, 17].

Micrnas (miRNA) plays an important role in regulating many biological pathways, such as innate immune response against various infections. recently, mirna have been studied as new potentials diagnostic biomarkers that are involved in several cases such as cancer, autoimmune disease and many

infectious diseases, including tuberculosis [18–28]. SNPs variants such as rs3746444 in miR-499, rs1799782 in miR-138 and rs1799782 in miR-146a may influence the pattern of gene expression related to the immune response and consequently influence in the genetics of different infectious diseases [22, 27, 29–31].

The bacillus M. tuberculosis is present worldwide and most of the people who fall ill with TB live in low- and middle-income countries. In the Americas, incidence is slowly increasing, particularly an upward trend in Brazil [32]. Brazilian population genetic structure reveals a high degree of admixture [33] due to this fact it is important in case studies and control to perform genomic control of ancestry [34–38].

In this study, we aimed to investigate the association of INDEL and SNPS variants for the susceptibility of Tb in an Amazon region with a high degree of interethnic admixture.

Methods

Subjects

A cohort of 428 participants were recruited: patients with active pulmonary TB ($n = 283$) and healthy controls without tuberculosis infection ($n = 145$). The subjects investigated were from the Hospital Universitário João de Barros Barreto in Brazil. Eligibility criteria for TB patients included: 1) ≥ 18 -year-old; 2) diagnosis of TB based on sputum smear examinations for acid-fast bacilli and/or the culture of *M. tuberculosis*, 3) started treatment until 6 months to signal and symptoms appeared. Control subjects were asymptomatic hospital volunteers with contact of bacteria-confirmed active TB patients, attending hospital for annual physical examination and no history of TB. Individuals from control group who contracted the tuberculosis disease were excluded from the study. Personal information was collected from a questionnaire including age, gender, comorbidities (cancer, diabetes, non-drug hepatitis and autoimmune disease - type 1 diabetes and rheumatoid arthritis), socioeconomic level and smoking habits.

Ethical approval

All protocols were approved by the João Barros Barreto Hospital ethics committee and the written informed consent was obtained from all participants involved in this study (protocol nº 350507).

Genotyping of selected polymorphisms

It was chosen seventeen representative genetic markers involved in different stages of the immune response (Additional file 1). Genomic DNA was isolated from peripheral blood leukocytes using phenol extraction method [39].

Four SNPs were genotyped using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, USA). Thirteen INDELs polymorphisms were genotyped by a single multiplex reaction with Master Mix QIAGEN® Multiplex PCR kit (Qiagen, Hilden, Germany) and the primers. Multiplex PCR products were

separated and analyzed by capillary electrophoresis on the ABI 3130 Genetic Analyzer instrument (Applied Biosystems), using GS-500 LIX as pattern of molecular weight (Applied Biosystems), G5 virtual filter matrix and POP7 (Applied Biosystems). After data collect, samples were analyzed in GeneMapper®3.7 software (Applied Biosystems).

Ancestry

Genetic admixture in the Brazilian samples were estimated using a set of 61 autosomal ancestry informative markers (AIMs). The protocol used was previously described by Santos *et al.* 2010 and Ramos et al [40, 41]. It was performed to estimate the mean contributions of European, African, and Native American ancestries to the Brazilian samples, using the STRUCTURE 2.3.3 software [42–44].

Statistical analyses

Statistical analyses were performed using the RStudio v.1.3 software. Group comparisons for categorical variables were performed using the chi-squared test, whilst the t-Student test was used to analyze the continuous variables. Multiple logistic regression analyses were performed to estimate the odds ratio (OR) with 95% confidence intervals (CI). Only the covariates that were significant (gender, comorbidities, smoking, ancestrality genetics – African, European and Amerindian) were included in the logistic regression analysis. A 5% significance level was used for the analyses. Deviation from Hardy-Weinberg equilibrium was assessed using chi-squared tests with Bonferroni correction. The results were analyzed comparing the genotypic distributions adopting the recessive and dominant model and they estimated values of *p* corrected for multiple tests through FDR (False Discovery Rate) test.

Results

The data of clinical and demographic distribution of tuberculosis patients and healthy individuals is shown in Table 1. The significant variables between the case and control groups were: The male patients were more frequent among tuberculosis patients (47.7%). In relation to comorbidity, the case group had higher rate than the control group (35.8%). Regarding smoking, among the patients the proportion of smoker was higher than in the control group (31.7%). Analysis of ethnicity showed that the mean frequency of Amerindian was higher among tuberculosis patients (0.336) and Europeans were more frequent in healthy individuals (0.496), there is also a significative difference of Africans but smaller than the others (0.253). Due to the great stratification of the Brazilian population, particularly the northern population, ancestry will be used as a confounding variable in risk analyzes.

Table 1: Demographic and clinical characteristics of the sample of patients with tuberculosis and of the control group without active tuberculosis.

VARIABLES	TUBERCULOSIS		<i>p</i> VALUE
	n = 283 (%)	CONTROL n = 145 (%)	
Age, years ^t	50 ± 16.84	52 ± 9.15	0.210
Gender ^b (M / F)	116 (47.7 %) / 127 (52.2%)	25 (17.3 %) / 120 (82.7%)	3.6E ⁻⁹
Comorbidities ^b	107 (35.8%)	67 (46.2%)	0.021
Smoking (Yes) ^b	77 (31.7%)	30 (20.7%)	0.002
^cAncestrality Genetics			
African	0.253 ± 0.12	0.215 ± 0.11	0.0002
European	0.409 ± 0.13	0.496 ± 0.16	5.7E ⁻⁹
Amerindian	0.336 ± 0.13	0.289 ± 0.14	0.00004

^t-Test Student T; ^b Fisher's Exact Test; ^c Mann Whitney Test; Data are shown as mean + - standard deviation.

The data obtained demonstrate that the polymorphisms investigated in the groups (patients and controls) had the genotypic distribution as predicted by the Hardy- Weinberg equilibrium. The findings present (table 2) the allelic and genotypic distribution relative to the 13 INDEL-type polymorphisms and the 4 SNPs investigated between the patient groups and the control group of the sample and the odds ratio (OR) among the groups analyzed.

Table 2: Association between groups for the 17 polymorphisms investigated.

GENE	PATIENTS n (%)	CONTROLS n (%)	p^c	OR (95 % CI) ^d
<i>IL1A</i> (rs3783553)				
INS / INS	64 (23.2%)	30 (20.7%)	0.844	0.934 (0.472-1.847)
INS / DEL	128 (46.4%)	70 (48.3%)		
DEL / DEL	84 (30.4%)	45 (31.0%)		
INS	0.46	0.45		
DEL	0.54	0.55		
<i>TYMS</i> (rs151264360)				
INS / INS	53 (19.2%)	14 (09.7%)	0.202	1.719 (0.78-3.950)
INS / DEL	120 (43.5%)	66 (45.5%)		
DEL / DEL	103 (37.3%)	65 (44.8%)		
INS	0.41	0.32		
DEL	0.59	0.68		
<i>UGT1A1</i> (rs8175347)				
INS / INS	116 (42.6%)	65 (46.1%)	0,132	0.978 (0.950-1.007)
INS / DEL	115 (42.3%)	55 (39.0%)		
DEL / DEL	41 (15.1%)	21 (14.9%)		
INS	0.64	0.66		
DEL	0.36	0.34		
<i>CYP2A1</i> (rs17880560)				
INS / INS	241 (86.3%)	120 (82.8%)	0.101	1.842 (0.887-3.825)
INS / DEL	37 (13.3%)	25 (17.2%)		
DEL / DEL	1 (0.40%)	0 (0%)		
INS	0.93	0.91		
DEL	0.07	0.09		
<i>SGSM03</i> (rs56228771)				
INS / INS	108 (39.1%)	74 (51.0%)	0.326	0.752 (0.427-1.327)
INS / DEL	122 (44.2%)	57 (39.3%)		
DEL / DEL	46 (16.7%)	14 (9.7%)		
INS	0.61	0.71		
DEL	0.39	0.29		
<i>IL4</i> (rs79071878)				
INS / INS	43 (15.6%)	21 (14.5%)	0.631	0.829 (0.384-1.786)
INS / DEL	133 (48.2%)	68 (46.9%)		
DEL / DEL	100 (36.2%)	56 (38.6%)		
INS	0.4	0.38		
DEL	0.6	0.62		
<i>MDM2</i> (rs3730485)				
INS / INS	18 (6.5%)	11 (7.6%)	0,174	0.457 (0.148-1.412)
INS / DEL	122 (44.2%)	58 (40.0%)		
DEL / DEL	136 (49.3%)	76 (52.4%)		
INS	0.29	0.23		
DEL	0.71	0.72		
<i>ACE</i> (rs4646994)				
INS / INS	17 (6.0%)	11 (7.6%)	0.005	0.465 (0.272-0.794)
INS / DEL	120 (42.4%)	49 (33.8%)		
DEL / DEL	146 (51.6%)	85 (58.6%)		
INS	0.27	0.25		
DEL	0.73	0.75		
<i>CCR5</i> (rs333)				
INS / INS	1 (0.4%)	0 (0.0%)	0.413	0.680 (0.270-1.712)
INS / DEL	18 (6.5%)	15 (10.3%)		
DEL / DEL	257 (93.1%)	130 (89.7%)		

INS	0.03	0.05		
DEL	0.97	0.95		
<i>UCP2 (no rs)</i>				
INS / INS	131 (47.5%)	74 (51.0%)		
INS / DEL	113 (40.9%)	57 (39.3%)	0.361	0.774 (0.447-1.340)
DEL / DEL	32 (11.6%)	14 (9.7%)		
INS	0.68	0.71		
DEL	0.32	0.29		
<i>HLA-G (rs371194629)</i>				
INS / INS	77 (26.0%)	49 (33.8%)		
INS / DEL	144 (48.6%)	72 (49.6%)	0.635	0.866 (0.478-1.568)
DEL / DEL	75 (25.3%)	24 (16.6%)		
INS	0.44	0.58		
DEL	0.56	0.41		
<i>XRCC1 (rs3213239)</i>				
INS / INS	13 (7.4%)	10 (6.9%)		
INS / DEL	88 (50.0%)	56 (38.6%)	0.109	0.390 (0.123-1.234)
DEL / DEL	175 (42.6%)	79 (54.5%)		
INS	0.21	0.26		
DEL	0.79	0.74		
<i>TP53 (rs17880560)</i>				
INS / INS	196 (71.0%)	101 (69.7%)		
INS / DEL	74 (26.8%)	42 (29.0%)	0.610	1.64 (0.649-2.087)
DEL / DEL	6 (2.2%)	2 (1.4%)		
INS	0.84	0.84		
DEL	0.16	0.16		
<i>miR-146A (rs2910164)</i>				
CC	128 (46.6%)	67 (47.9%)		
GC	123 (44.7%)	57 (40.7%)	0.256	1.753 (0.666-4.617)
GG	24 (8.7%)	16 (11.4%)		
C	0.69	0.68		
G	0.31	0.32		
<i>miR-192A (rs11614913)</i>				
TT	23 (8.4%)	9 (6.7%)		
CT	119 (43.4%)	55 (40.7%)	0.113	1.578 (0.888-2.773)
CC	132 (48.2%)	71 (52.6%)		
T	0.3	0.27		
C	0.7	0.73		
<i>miR-499 (rs3746444)</i>				
GG	21 (7.4%)	10 (6.9%)		
GA	111 (39.2%)	55 (37.9%)	0.166	0.717 (0.447-1.149)
AA	151 (53.4%)	80 (55.2%)		
G	0.27	0.26		
A	0.73	0.74		
<i>miR-149 (rs2292832)</i>				
CC	36 (13.2%)	19 (13.2%)		
TC	115 (42.3%)	66 (45.8%)	0.206	0.639 (0.320-1.279)
TT	121 (44.5%)	59 (41.0%)		
C	0.34	0.36		
T	0.65	0.63		

^c p-value obtained by regression age-adjusted logistics, gender, comorbidity, smoking, and genetic ancestry. Adopting the dominant model. The p values were corrected for FDR multiple test; ^d *Adjusted Odds Ratio (OR)*.

When it was investigated INDELS and SNPs polymorphisms, only the DEL/DEL genotype of the *ACE* variant (rs4646994) showed significant relative risk between cases and controls (OR = 0.465; p = 0.005). The analysis of this marker showed that in the dominance test (DEL / DEL genotype vs INS / DEL + INS / INS), there was a greater proportion of homozygous individuals DEL / DEL among the control subjects (51,6%) compared to the patients (51,6%). The Odds Ratio of 0.465 indicates protective effect in individuals with DEL/DEL genotype in relation to the control group.

Discussion

TB is a complex disease, and it is known that the host and pathogen factors are related to the severity in the TB pathogenesis [45, 46]. Several studies provide evidence that genetic factors play a major role in toward progression of TB, evidencing that polymorphisms in genes can influence and modulate the immune responses and this would be one of the reasons why infected people with *Mtb* are not progressing to TB disease [28, 37, 47].

In this investigation, of the case-control type genetic association studies in admixed populations, ancestry-informative markers was used to detect population stratification. Ancestry related data showed significantly different contribution between groups for African, European and Native American ancestry. These results show a loss of European contribution and an increase of African and Native American contributions in patient group with Tb. A recent study of our group had already shown that there are differences between case and control groups about ancestry: the individuals with TB disease had significantly higher Amerindian ancestry and significantly lower European ancestry in Brazilian Amazon population [33].

Results showed that only the polymorphisms in the *ACE* gene were significant for the analysis of susceptibility to TB in the investigated groups regarded to the 17 genes investigated. We found DEL/DEL genotype of *ACE* marker (rs4646994) showed significant association by setting up a protective factor for the development of tuberculosis. We sought to interpret these findings based on the genotype-dependent expression of this enzyme and on its ability to stimulate innate immunity from macrophages and neutrophils against mycobacteria[48] .

ACE has a role in both innate and adaptive responses by modulating macrophage and neutrophil function, effects that are due to increased *ACE* activity [48–51]. Recent studies point to the functionality of *ACE* in promoting the maturation of myelocytic lineage cells and to improve the effective phagocytosis capacity and consequent self-limitation of *Mtb* infection [52, 53]. In this context, individuals with DEL/DEL genotype would have greater expression of the gene that would have a crucial role in the innate effective immune response, mainly because the control group was exposed to the bacillus without developing the disease.

Because of its role in immune response, the rs4646994 is mostly related with the susceptibility of diseases. A study also with an admixed population in Brazil shown that this polymorphism was associated with Colorectal Cancer risk and clinical features. It is also related with the progression of

multiple myeloma in a Caucasian population and the development of moderate-sized adenocarcinomas and metastatic cancer in a Chinese population [54, 55].

Recent studies associated the DEL/DEL genotype with the risk of developing severe COVID-19 and increasing morbidities, this polymorphism also was associated with the progression of pneumonia in Severe Acute Respiratory Syndrome (SARS), corroborating its importance in the development of contagious infectious diseases [56–58].

There are few studies associating the DEL/DEL genotype with tuberculosis, yet *Ogarkov and contributors* in 2008, shown that this polymorphism was more frequently in male patients with tuberculosis in a Russian population, diverging from our results [59]. Also not consistent with our results, a study in a Chinese population demonstrated that the rs4646994 was not a major etiological factor for tuberculosis [26].

Conclusion

From the data obtained in our study is possible to conclude that individuals with DEL/DEL genotype of the rs4646994 in ACE gene presented a 46% protective effect against the development of tuberculosis than individuals with other genotypes (OR = 0,46). Nevertheless, as there are few studies with susceptibility to Tb in a Brazilian population, more studies are necessary with our population, once Brazil still remains among the 20 countries with more incidence of Tb.

List Of Abbreviations

PB: base pair; *ACE 2*: angiotensin-converting-enzyme 2; AIMS: ancestry-informative markers; *CCR5*: chemokine receptor type 5; CI: confidence intervals; *CYP2E1*: Cytochrome P450 2E1; DEL: deletion; FDR: False Discovery Rate; *HLA-G*: Human leukocyte antigen-G; *IL 1A*: interleukin 1 alpha; *IL 4*: interleukin 4; INDEL: insertion/ deletion; INS: insertion; *MiR*: miRNA or microRNA; *MDM2*: Mouse double minute 2 homolog; *Mtb*: *Mycobacterium tuberculosis*; NF-KB 1: Factor Nuclear Kappa B; OR: odds ratio; RNA: ribonucleic acid; SNP: Single nucleotide polymorphisms; *SGSM03*: *Small protein G signaling modulator*; TB: Tuberculosis; *TGF-β*: Beta growth transformation factor; *TP53*: tumor protein p53; *TYMS*: thymidylate synthase; *UCP2*: mitochondrial uncoupling protein 2; *UGT1A1*: UDP Glucuronosyltransferase Family 1 Member A1; WHO: world health organization; *XRCC1*: X-ray repair cross-complementing protein 1.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the João Barros Barreto Hospital ethic committee and followed the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. The patients signed an informed consent before entering the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are available in the Figshare repository, <https://figshare.com/s/0af7c9a15996be56a062>.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

DCRFP, DFVBL, MNSS, GEC and MCCB prepared the manuscript and interpreted the data; ACOB, CAS and PPA contributed for the data collection; PDCP, LCBS and PDCP conducted statistical analysis; PPA, ARS, SEBS, MRF and NPCS contributed to study design, reviewed and edited the manuscript. All authors have revisioned, read and approved the manuscript

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