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The D543N polymorphism of the *SLC11A1/NRAMP1* gene is associated with treatment failure in male patients with pulmonary tuberculosis

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Abstract

Background: Polymorphisms in *SLC11A1/NRAMP1* have shown an important association with susceptibility to tuberculosis and progression to active disease. However, whether there is an association of these polymorphisms with treatment failure is unknown. The aim of this study was to determine the association of *SLC11A1* polymorphisms with treatment failure in Mexican subjects with pulmonary tuberculosis.
Methods: Thirty-three subjects with treatment failure were paired by age and body mass index with 33 patients who successfully completed treatment and were considered cured. We assessed the polymorphisms of *SLC11A1* in the regions of D543N and INT4 via polymerase chain reaction real-time TaqMan® single nucleotide polymorphism (SNP) genotyping.
Results: We found that D543N (G/A genotype) was associated with treatment failure in patients with pulmonary tuberculosis [odds ratio (OR) 11.61, 95% confidence interval (CI) 3.66–36.78]. When adjusted by gender, this association remained significant in males (OR 11.09, 95% CI 3.46–35.51).
Conclusions: In our male population, the presence of the D543N polymorphism of *SLC11A1* is a risk factor for treatment failure. This finding should be confirmed in other populations.

Keywords: pulmonary tuberculosis; *SLC11A1/NRAMP1*; treatment failure.

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Introduction

Tuberculosis (TB) remains one of world's deadliest infectious diseases. However, the World Health Organization (WHO) estimates that most deaths from TB are preventable with early recognition and appropriate treatment.

The growth of *Mycobacterium tuberculosis* (MTB) is usually inhibited by the development of an effective immune response. Therefore, only approximately 10% of infected subjects will eventually develop clinical disease.

In 2013 in Mexico, there were 19,738 new cases of pulmonary TB (PTB). In the states, where this study was performed, approximately 1300 naïve treatment subjects received therapy, which failed in 58 subjects [1].

The progression of PTB is strongly associated with immunosuppression. However, this is not a universal finding, suggesting that other factors, both environmental and host dependent, are involved [2]. The latter case is exemplified by the MTB Beijing genotype, which is strongly associated with polymorphisms in the *SLC11A1/NRAMP1* gene [3].

The SLC11A1-encoded product is an integral membrane protein that is exclusively expressed in the lysosomal compartment of monocytes and macrophages, acting as a pump for divalent cations (Fe^{2+} and Mn^{2+}) from the extracellular milieu into the cytoplasm of a macrophage.

After the generation of the phagosome, SLC11A1 removes Mn^{2+}, which is critical for the production of metalloenzymes by bacteria, thus preventing their multiplication [4]. The precise role of *SLC11A1* polymorphisms in TB disease is still controversial. Li et al. conducted a meta-analysis involving 36 studies on the relationship between TB and four polymorphisms, one of which, D543N, showed a significant association with TB susceptibility [5]. *SLC11A1* polymorphisms (D543N and INT4) also contribute to the severity of PTB through uncontrolled MTB growth [6]. At the individual level, the differences in the host immune response play critical roles in TB susceptibility and progression, but information about the association of polymorphisms in genes involved in immune control and the treatment failure is scarce [7]. Thus, the aim of this study was to determine the association of the D543N and INT4 polymorphisms in *SLC11A1* with treatment failure in patients with PTB.

Materials and methods

We conducted a case-control study in which the case group was defined as PTB-affected patients showing anti-TB treatment failure, whereas subjects with the same diagnosis, but with a favorable treatment response constituted the control group. Age and body mass index were the matching criteria. None of the patients had undergone TB treatment previously. All of the subjects received the first-line treatment recommended in Mexico for newly diagnosed patients: isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months, followed by 4 months of isoniazid and rifampicin. All cases of PTB were bacteriologically confirmed through culture of microscopic evaluation (three positive smears).

The Ethics and Research Committees of the participating institutions approved the study protocol. All participating subjects signed an informed consent form and answered a questionnaire.

The target population consisted of 66 TB patients (41 men and 25 women) aged 33–66 years (mean=49 years) from the Department of Epidemiology of the Ministry of Health, Mexican Institute of Social Security (IMSS) of Durango and Veracruz, Mexico.

All patients presented PTB symptoms (WHO 2014) and exhibited a positive culture or acid-fast bacilli in their sputum samples. Qualified personnel at each health unit performed sputum smears. A pulmonologist and/or an epidemiologist diagnosed active disease.

Treatment failure was defined as a patient whose sputum smear was positive in two consecutive months or showed a positive culture at month 6 or during treatment.

Response to treatment was defined as the disappearance of clinical signs of PTB and three negative sputum smears in two consecutive months or a negative culture at the end of treatment in an individual.

Genotyping was performed with the StepOne™ real-time PCR system (Applied Biosystems®, Carlsbad, CA, USA) using the probes TaqMan® MGB C_25635296_10 and C_1659793_10 for identification of the exonic SNP rs17235409 G>A (D543N) and the intronic SNP rs3731865 G>C (INT4), respectively.

Differences between groups were assessed with the unpaired Student's t-test for numerical variables and the χ^2-test for nominal variables. The frequencies of different genotypes were obtained via direct counting, and Hardy-Weinberg equilibrium (HWE) was calculated using the χ^2-goodness-of-fit statistic; these analyses were carried out with SNPstats (http://bioinfo.iconcologia.net/SNPstats). The association between polymorphisms and treatment failure was evaluated with a logistic regression analysis model. The odds ratio and p-value were calculated for the alleles and genotypes in the patient and control groups. A 95% confidence interval (95% CI) was considered, and a two-tailed p-value <0.05 defined the level of statistical significance. Statistical analysis was performed using SPSS V.17.0 (SPSS Inc, Chicago, IL, USA).

Results

A total of 66 patients with PTB were included, 33 of whom exhibited treatment failure. There were no significant basal differences between the cases and controls (Table 1).

The frequency of allele C for the rs3731865 SNP did not show significant differences in the case and control groups, whereas the frequency of allele A for the rs17235409 SNP was significantly higher in the case than in the control group (p<0.001).

The frequency of the G/G genotype (SNP rs17235409) was significantly higher in the control group than in the case group (p<0.001). Conversely, the G/A (p=0.01) and A/A (p=0.02) genotypes presented a higher frequency in the case group compared with the controls.

The population in the controls and cases was in HWE for both the rs17235409 (p=0.082 and p=0.073, respectively) and rs3731865 (p=1 and p=0.23, respectively) SNPs.

Crude multivariate regression analysis showed that SNP rs17235409 (OR 11.61, 95% CI 3.7–36.8, p<0.0001), but not SNP rs3731865 (OR 0.88, 95% CI 0.33–2.38, p=0.8), was

Table 1: Study cohort.

Variable	Case 33	Control 33	p-Value
Male, n (%)	23 (70)	18 (55)	0.21[a]
Female, n (%)	10 (31)	15 (45)	
Age, years	49.7±16.1	49.6±15.8	0.90[b]
Weight, kg	58.0±16.3	60.9±15.0	0.15[b]
Height, m	1.6±0.09	1.5±0.09	0.37[b]
Body mass index	21.5±4.8	23.9±5.7	0.07[b]
Diabetes type 2, n (%)	16 (48)	11 (33)	0.21[a]
Smoking, n (%)	2 (6)	7 (21)	0.07[a]
Drug, n (%)	1 (3)	6 (18)	0.06[a]
Alcohol, n (%)	4 (12)	5 (15)	0.72[a]
COMBE, n (%)	16 (49)	21 (64)	0.22[a]

Values are means±SD unless otherwise indicated. n, number.
[a]χ^2-test. [b]Mann-Whitney U-test.

significantly associated with treatment failure, in agreement with a dominant inheritance model.

The analysis of SNP rs17235409 revealed that men in the case group exhibited a significantly higher frequency of allele A (58%) than men in the control group (12%) ($p<0.0001$) (Table 2). Multivariate regression analysis showed a strong association between this SNP (genotype G/A) and treatment failure (OR 11.09, 95% CI 3.46–35.5, $p<0.0001$), which agrees with a dominant inheritance model. On the other hand, the analysis in women did not show any association.

Discussion

Our results suggest that the rs17235409 SNP is associated with treatment failure in male patients with PTB. We believe that this association is favored by impaired host immunity more than by drug resistance development.

PTB treatment failure constitutes a source of infection, disease transmission, and spreading of potentially resistant mycobacteria. Drug resistance, non-adherence, and prior treatment have been described as causes for treatment failure [8, 9]. The WHO considers that among newly diagnosed cases, the rate of MTB resistance is 3.5% globally. The frequencies of resistance at the sites where the patients were enrolled were 25% and 5%. However, this information includes both new and previously treated subjects. Therefore, we believe that resistance to MTB is quite similar to WHO estimates. Consequently, the absence of a susceptibility test is only a partial limitation of this study. Recently, Yang et al. demonstrated a lack of association between a drug-resistant Beijing strain and treatment failure [10]. Susceptibility to MTB infection is determined in part by the genetic-associated host response to disease progression. van Crevel et al. [3] showed a significant association between the D543N mutation in the *SLC11A1* gene with a Beijing strain of MTB. This suggests a potential association between disease progression and treatment failure mediated through *SLC11A1* gene function.

This study was not designed to evaluate the association between *SLC11A1* polymorphism and susceptibility to PTB or disease progression because all subjects included in the study exhibited active TB. Susceptibility to disease and progression only can be proven with the inclusion of both healthy and latent TB subjects.

Genetic studies on the treatment response in the case of infectious diseases must include the evaluation of genes involved in host-pathogen interactions. In this study, the analysis of two polymorphisms, INT4 and D543N, in *SLC11A1* gene demonstrated a strong association between the mutant allele of the D543N polymorphism (rs17235409) and treatment failure in men. This finding could be explained by the stronger immune responses to infection and vaccination observed in women compared with men [11]. These observations suggest large hormonal influences on the treatment response; however, this is out of the scope of this work.

Relevant polymorphisms in *SLC11A1* such as 3′UTR and 5-(CA)n were not evaluated in this study, which should be considered to design additional studies of association with treatment failure in TB patients.

Treatment failure is significantly more common in men, as demonstrated in a study conducted in Morocco [12]. Furthermore, polymorphisms in the *SLC11A1* gene were found to be associated with active TB in a male Tunisian population [13]. Our study not only confirmed the results obtained in Morocco and Tunisia but also identified a strong association between the rs17235409 SNP (11.09, 95% CI 3.46–35.51) and treatment failure in males.

In conclusion, we found that the G/A genotype of the D543N polymorphism of *SLC11A1* in male patients with PTB was associated with treatment failure. Larger multicenter studies are needed to confirm the results of this study.

Table 2: Allele and genotype frequencies for SNP rs17235409 (D543N) among cases and controls by gender.

	Male			Female		
	Case n=23, n (%)	Control n=18, n (%)	p-Value[a]	Case n=10, n (%)	Control n=15, n (%)	p-Value[a]
G	19 (42)	32 (88)	<0.0001	12 (60)	25 (84)	0.65
A	27 (58)	4 (12)		8 (40)	5 (16)	
G/G	3 (13)	15 (84)	<0.001	5 (50)	11 (74)	0.23
G/A	13 (57)	2 (12)	0.002	2 (20)	3 (20)	1.0
A/A	7 (30)	1 (4)	0.038	3 (30)	1 (6)	0.11

[a]χ^2-test.

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